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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

Fiscal year 1972 witnessed moderate growth of the National Institute of Environmental Health Sciences. Plans are developed to fill in some of the remaining gaps in staffing, facilities, and other resources. Programs of the Institute were strengthened significantly, especially through the creation of the Mutagenesis Branch under Dr. Frederick de Serres and the addition of Dr. Robert L. Dixon as Branch Chief of the Pathologic Physiology Branch, with the subsequent broadening of the Branch's program.

FACILITIES

The completion of the addition to the interim facilities made possible the development of a program in aerosol toxicology. The installation of some exposure chambers has been completed and experiments are in progress. The new animal building has afforded the Animal Science and Technology Branch the opportunity to improve the environment of experimental animals, and consequently the quality of the animals in the Institute's research program, and has met the growing need for space in which to conduct animal studies.

The Research Services Branch, in collaboration with the Office of Engineering Services, NIH, has prepared the program of requirements for the first stage of construction of the permanent site. These documents define the program to be conducted in the buildings and outline the engineering and architectural features of the facilities. Following apportionment of the planning funds by the Office of Management and Budget and the selection of an architect-engineering firm by HEW, design of the buildings and utilities will proceed.

CONSULTATION AND LIAISON

This year the liaison activities of the Institute have related largely to its scientific program. The Director and members of his staff have continued to serve as advisors to the Office of the Secretary, HEW, and to represent the Department in activities concerned with current environmental problems, ranging from detergents to dielectric fluids and plasticizers. In addition, the NIEHS has cooperated with the Food and Drug Administration in planning the research programs of the National Center for Toxicological Research.

In addition to consultation and liaison, the Institute works with both permanent and *ad hoc* commissions, task forces, and technical committees. These activities

have proven to be effective mechanisms for exchange of information on the broad problems common to the several agencies.

The Director was appointed Chairman of an OST-CEQ Ad Hoc Advisory Committee on Environmental Research in October 1971. Its charge was to survey the various environmental research activities and information sources within the Federal structure, to determine the adequacy of this structure with respect to regulatory decisions in public health, and to recommend mechanisms whereby adequate coordination may be developed and continued. A presentation of the major findings was made to PSAC in May. The Committee's report was sent to OST and CEQ in June.

The Associate Director for Scientific Information and Communication represented DHEW on a parallel Committee on Ecology reporting to CEQ and FCST. An Inventory of Federal Research in Ecology was sent to CEQ in June. The final report will be sent to CEQ in September.

In collaboration with the Fogarty International Center, four books have been prepared on selected environmental health problems: (1) Metallic Contaminants and Human Health; (2) Mutagenic Effects of Environmental Contaminants; (3) Environmental Factors in Respiratory Disease; and (4) Multiple Factors in the Causation of Environmentally Induced Disease. The first three have been published by Academic Press. The material was prepared in each case by a panel of contributors that met for intensive discussion at the Stone House, NIH Subsequent editing was done by NIEHS and FIC staff.

PCB CONFERENCE

With the recognition of the wide-spread distribution of polychlorinated biphenyls (PCBs) in the environment, including the food chain, and the lack of information on the effect of these compounds on man's health, the Institute sponsored a conference on December 20-21, 1971, in North Carolina, to assess the state of knowledge about PCBs and their impact on human health. Participants included representatives of industry, control and regulatory agencies, and the press, as well as scientists working on the problem. The proceedings of the conference were published in the first experimental issue of the Institute's journal, ENVIRONMENTAL HEALTH PERSPECTIVES, in May.

INTERNATIONAL PROGRAMS

The Director has continued to participate in the planning of environmental studies as a part of the U.S. - Japan Cooperative Medical Sciences Program. At a meeting on January 20-21, 1972, the Joint Subcommittee on Program Review and Planning recommended that a symposium or mutagenic and carcinogenic effects of environmental pollutants be convened in Tokyo on August 25-26, 1972. A Panel on Methods to Evaluate Environmental Mutagenesis and Carcinogenesis has been established with Dr. Frederick de Serres as Chairman. The primary function of this group is to develop methods and test systems to determine how chemicals and other environmental agents are related to the pathogenesis of human cancer or mutagenic effects in man.

Dr. Edward E. David, OST, requested the Director to play a major role for the U.S. in establishing a United States - United Kingdom collaborative program for research in environmental sciences. At an exploratory meeting in Washington, D. C. on March 20, 1972, with Sir Allan Cottrell, Chief Science Advisor to Her Majesty's Government, four general topics were agreed upon for future cooperation--predictive tests, safety criteria, criteria for pathological diagnoses, and chronic disease epidemiology.

During the week of March 25-31, 1972, the Director participated in the meeting of the new Joint Committee on Health Cooperation of the United States - Soviet Health Exchange in Moscow, U.S.S.R. As a first step in establishing a program of scientific collaboration between the Institute of General and Community Hygiene, AMS, U.S.S.R., and the NIEHS, three problems of mutual interest were identified: (1) methodological basis of the assessment of the biological effect of inhaled chemicals; (2) methodological basis of the assessment of the biological effect of orally introduced chemicals; and (3) scientific basis of the assessment of the complex biological effect of chemicals (inhaled and introduced orally). In October 1972, specialists from the Soviet Union will visit NIEHS in North Carolina to develop the details of the program.

SUBCOMMITTEE HEARING

On April 24, 1972, the Intergovernmental Relations Subcommittee of the House Committee on Government Operations held a hearing in Raleigh, North Carolina, on the work of the Institute with respect to environmental problems which may endanger the Nation's health. Representative L. H. Fountain (D-NC) headed the Subcommittee hearing to learn how the Institute relates to other governmental agencies in the same field and to discuss the scientific knowledge and capabilities needed to combat environmental health hazards. Following the hearing, the Subcommittee toured the Institute facilities and was further briefed on its activities.

COMMUNITY ACTIVITIES

Collaborative and cooperative efforts with the Triangle universities have achieved maturity during the year. Undergraduate and graduate teaching, graduate student training, and joint research efforts between intramural scientists and university staff have grown steadily to the mutual benefit of the NIEHS and the Triangle universities.

In summary, fiscal year 1972 has been a year of continuing program development and growing responsibility in Federal science activities. The coming year should bring continuing program maturation and the development of fullscale research programs in areas where only modest efforts were heretofore possible.

OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM
Summary Statement

During fiscal year 1972, the Office of the Associate Director for Program initiated multiple research programs to clarify toxicological problem involving heavy metals. Several major research programs dealing with the origin, the distribution, the interaction and the fate of metals in man's environment were activated through contracts. These contracts will also pay special attention to toxicological hazards of heavy metal complexes.

In a similar thrust, a study has been initiated on organic industrial chemicals which promise to be widely distributed or have already reached large-scale distribution. The toxicity of these compounds for man and his environment must be studied, and this is particularly true for those compounds which were found unreactive and not degradable in mammalian species or the general environment. An effort has been made to develop more competency in this area of technological surveillances, and use will be made of the National Academy of Science's Presidential Internship in Science and Engineering Program to obtain competent graduates in chemical engineering. These interns will contribute their knowledge to this program and gain, in exchange, training in problem solving relating to environmental health. These activities will be coordinated with the activities of intramural scientists to obtain the greatest amount of scientific input and relevance. The program will have its emphasis on health hazards that may arise in the near future and does not pretend to be able to project technological advances many years into the future. This program has been discussed with Dr. Chapin of the National Science Foundation so that it will work as smoothly as possible.

As in previous years, this office has been active in giving constructive and critical advice to U. S. Governmental agencies on a number of important documents prepared by them. Most of these requests came from the Environmental Protection Agency and the U. S. Department of Agriculture. One of these tasks dealt with the revision of the Federal Drinking Water Standards of 1971. In addition, the requirement of the National Environmental Policy Act for Governmental agencies to prepare environmental impact statements for all major action programs under consideration has lead to several requests for advice on these impact statement drafts, particularly regarding pesticide usage. Environmental impact statement drafts involving the control of the gypsy moth, the spruce bud worm and grasshopper invasions, the safe disposal of the pesticide organge, the problems regarding the effect of noise produced by supersonic aircraft and the hazard of radar exposures have been reviewed and then forwarded to the Surgeon General for evaluation. For some of these critiques, advice was solicited from other members of the

Institute. Reports from the Task Force on Training and Objectives and Standards, and the Task Force on Categorization of Pesticides used by the Federal Government were also critically reviewed.

On request by the Subcommittee on Intergovernment Relations of the House Committee on Government Operations, the Associate Director accompanied the Director of the Institute to a hearing before this committee in order to answer questions regarding carcinogenic hazards for man. This Office also responded to questions from a Senate Commerce Committee regarding potential carcinogenic risks from inhalation of freons.

On request by the Government Accounting Office, the Associate Director explained in detail reasons or opinions regarding the actions recommended or taken by EPA which would curtail the use and distribution of a number of specific pesticides of particular interest to that office.

During the fiscal year, the Associate Director of Program participated in many activities regarding health problems on an international basis. He prepared a number of documents for the International Agency for Research on Cancer on the carcinogenic risk for man due to exposure to specific metals and helped to extrapolate the evidence of carcinogenic activity in animal experiments to the human experience. This program is particularly important when extrapolation of laboratory data for suspect carcinogens is required because human epidemiologic data is entirely missing. This activity is an on-going commitment, and members of this IARC committee meet annually to see whether agreement can be reached on all recommendations submitted during the year on the various carcinogenic evaluations.

At this time, a preamble for a monograph on the topic of the carcinogenicity of polycyclic aromatic hydrocarbons for man is being prepared by this office for the IARC. The Associate Director was also asked to attend a meeting of the Advisory Committee on Environmental Carcinogens of the IARC to help in deliberations on quantitative aspects of carcinogenesis, with special emphasis on hazards due to low-level exposures.

On request by the Director of the Division of Environmental Health of the World Health Organization, the Associate Director spent several weeks in Geneva helping staff prepare various documents on environmental health which were used either in the WHO Monograph on Health Hazards of the Human Environment or as background documents for the conference to be held in Stockholm, Sweden. Since returning from this assignment, contact has been maintained by visits from various staff members of WHO, and requests for comments and revisions of later versions of the documents have been answered by mail.

The Associate Director, together with scientists of other Institutes of NIH, was also involved in discussions on cooperation in the US-Japan Collaborative Program regarding environmental hazards which deserve attention. During the year many visits were made by foreign scientists, and this office tried to supply the information desired or the services needed. Visitors from Japan wanted information on facilities for bioassay as new research buildings were being designed in Japan. Russian visitors were interested in the

environmental health program of the Institute. A scientist from Israel spent a week at the Center to familiarize himself with several aspects of research carried out at the Institute. The Director of the Department of Health of Victoria, Australia, came to discuss problems of environmental health; and similar discussions were held with the Director of the Institute of Toxicology, National Food Institute of Denmark, and with the Minister of Public Health from the Netherlands who visited the Center. An inquiry from the Secretary of Health of Mexico regarding detergent enzymes was also answered by this office.

Regarding tobacco and health, this office contributed discussions and evaluations to scientists representing the Research Institute of the German Tobacco Industry, the British American Tobacco Institute, the United States Department of Agriculture and local universities. The topics under discussion varied from health hazards due to pesticides and their degradation products to cocarcinogenic factors in tobacco and the meaning and extrapolation of such bioassay results. The Associate Director also prepared critical comments for the chapter on cancer in the document prepared by the National Clearing House for Smoking and Health.

With regard to air pollution and health, the Associate Director, on the urging of the National Academy of Science, agreed to participate in the preparation of a document on "Vapor Phase Organic Air Pollutants from Hydrocarbons." As a member of that committee, he prepared a chapter on the topic of "Synergistic and Inhibitory Effects" of these air pollutants and participated in meetings of the total committee during which this document was put together. This report will be used by the EPA in their deliberations regarding health hazards of automobile exhaust components.

On invitation by the National Cancer Institute, the Associate Director participated in the preparation of the National Cancer Plan. This was carried out at Airlie House in Warrenton, Virginia, during a three-day period. Subsequently, an evaluation of the total program was supplied to the NCI when sections of the National Cancer Plan had been put together.

Several discussions were held with members of industry and the Environmental Protection Agency on various problems relating to the safety of detergents. In one conference sponsored by EPA, the potential health hazard of optical brighteners was reviewed. Other discussions dealing with nitrilotriacetate (NTA) were held during the year with industry and the office of the Surgeon General.

The Associate Director was asked to attend the Fifth Annual National Control Conference and Exposition in Houston, Texas, to discuss health aspects, problems which had not been touched on by any of the other speakers at that conference. Also on invitation, he gave the opening lecture in a symposium on "Current Problems in Food-Borne Environmental Chemicals of Health Significance" sponsored by the American Chemical Society in their 163rd meeting held in Boston. Associated with this presentation was a lengthy press conference, during which many of the problems were discussed for the layman. The Associate Director also gave several lectures on topics varying

from carcinogenesis to the toxicity of environmental chemicals, like the PCB's, at various opportunities, particularly at Duke University. He also talked to several groups of visiting scientists with various backgrounds on problems of environmental health and on the mission and function of the NIEHS in comparison to other agencies of the Federal Government. In addition, he discussed problems regarding health that we may face tomorrow at a symposium on "A Better Tomorrow Through Technology."

The Associate Director was invited to prepare a chapter on "Chemical Carcinogens, Mutagens, and Teratogens" for a textbook entitled Environmental Problems in Medicine. This chapter has been accepted and will be in print in the fall of 1972. In order to bring environmental problems into the correct perspective, and experimental journal, Environmental Health Perspectives, was created and will be published quarterly. The first issue of this journal presents the proceedings of the PCB conference which was held under the auspices of this Institute. Subsequent issues will present invited papers on specific environmental health problems and their critical evaluation. Together with Dr. D. Lee, the Associate Director for Program serves as Associate Editor of this journal. He also serves on the editorial board of Toxicology and Applied Pharmacology, Environmental Letters, Chemico-Biological Interactions, as well as an occasional reviewer for Science, JNCI, and other journals.

Serial No: NIEHS-ADP-001
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "An Investigation of Synergism Between Degradation Products of Polyvinyl Chloride Manufacture and Common Environmental Agents With Regard to Porphyrogenesis"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Hans L. Falk, Ph. D.

OTHER INVESTIGATORS: Sandra Jordan

COOPERATING UNITS: None

MAN YEARS

Total	1.1
Professional:	0.1
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Hexachlorobenzene has been found to be porphyrogenic for animals as well as man when absorbed as a dietary contaminant. The process of its formation suggests that this compound may be formed as part of the action of microorganisms on compounds with carbon chlorine bonds. In this process other compounds are also formed which may affect the toxicity of hexachlorobenzene. Because these compounds have different target organs and produce different degrees of toxicity, it will be of significance to test combinations of these compounds as well as their effect in the presence of other common pollutants that can be expected to affect the porphyrogenic process.

METHODS EMPLOYED: Studies are done by feeding the compounds to rats and determining porphyria when it occurs. The duration of porphyria after the chemicals have been withdrawn from the diet is also determined. As lead will produce its porphyria by interfering with enzymes other than those involved in the action of hexachlorobenzene, a combination of these two agents should have a marked synergistic effect which will be investigated. Methods have been checked and modified to quantitate these fluorescent compounds in urine and feces. Animals have also been placed on hexachlorobutadiene, which is another PVC reaction side-product expected to be present, and these animals are now under observation.

MAJOR FINDINGS: Feeding experiments with hexachlorobenzene have produced porphyria in the animals. The duration of porphyria is under study as well as the length of feeding of the compounds required to re-establish porphyria in rats which had recovered from earlier porphyria.

SIGNIFICANCE: These compounds are under study because they are common pollutants of the environment which find their way into the food and thus exert their toxic reactions. Many of the compounds which have been studied for their adverse effect have been found to be microsomal enzyme inducers in the liver, and hexachlorobenzene induces δ-ALA synthetase, an enzyme instrumental in producing porphyria. Whether other common contaminants, like the PCB's or chlorinated hydrocarbon insecticides, augment this porphyrogenic effect needs to be established, because in humans the presence of these compounds (DDT, DDE, PCB's, benzenehexachloride, as well as hexachlorobenzene, etc.) has already been demonstrated in blood samples.

PROPOSED COURSE: These porphyrogenic studies will be continued to investigate synergism or antagonism between chemicals found in our environment, and efforts will be made to understand their reaction, to determine critical levels at which these interactions occur and to establish the duration of porphyria.

CONTRACTS

AMERICAN ACADEMY OF PEDIATRICS, EVANSTON, ILLINOIS

TITLE: "Early Detection of Environmental Agents that Induce Diseases in Children"

CONTRACTOR'S PROJECT DIRECTOR: Stanley L. Harrison, M.D.

PROJECT OFFICER (NIEHS): R. A. Tjalma, D.V.M.

DATE CONTRACT INITIATED: May 1, 1972

CURRENT ANNUAL LEVEL: $36,500

PROJECT DESCRIPTION

OBJECTIVES: Identification of environmental stressors which are associated with or induce birth defects or other disease conditions in newborn children. Major emphasis is directed to clinical identification of stressors which may have acted to induce congenital defects.

METHODS EMPLOYED: Three academic pediatric centers, located in California, New York and North Carolina, are participating in the study under the direct auspices of the American Academy of Pediatrics. Neonates with congenital defects and other diseases considered possibly attributable to environmental stress during intrauterine development are identified and a detailed study of the maternal and paternal environmental history is conducted. Particular emphasis is placed on documentation of maternal pregnancy history as regards use of therapeutic drugs, unusual dietary habits, occupational exposures and disease conditions; reproductive history and the occurrence of disease among family members are documented. Results from each pediatric center are summarized at regular intervals and submitted to the Committee on Environmental Hazards, American Academy of Pediatrics for review, evaluation and submission to NIEHS. Important clues are reported immediately to NIEHS by the pediatric staff of the individual centers and, when indicated, technical support is provided in an effort to identify the suspect stressor.

MAJOR FINDINGS: The study is in the process of organization.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developing embryo/fetus is unusually susceptable to toxic stress and insult and may well constitute a particularly sensitive indicator of environmental hazards. The study is designed to provide clinical clues to environmental stressors and leads for more specific laboratory studies.

PROPOSED COURSE: The study will be continued for the remainder of the initial year and evaluated at that time.

BATTELLE NORTHWEST-PACIFIC NORTHWEST LABORATORY, RICHLAND, WASHINGTON
(Interagency Agreement Between NIEHS and AEC)

TITLE: Research on the "Combined Effects of Inhalation of Uranium Ore Dust and Radon Daughters with Cigarette Smoke or Diesel Exhaust Fumes on the Lungs of Dogs and Hamsters"

CONTRACTOR'S PROJECT DIRECTORS: J. F. Park, Ph.D. and B. O. Stuart, Ph.D.

PROJECT OFFICER (NIEHS): Hans L. Falk, Ph.D.

ASSOCIATE PROJECT OFFICER: Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: June 1, 1968

CURRENT ANNUAL LEVEL: $331,795

PROJECT DESCRIPTION

OBJECIVES: As a major part of an integrated research program on the effects of exposure to radon and daughters on the development of lung cancer in uranium miners, the following animal studies were initiated:

1. Groups of hamsters were exposed to the following combinations:

   a. radon daughters on room air nuclei,
   b. radon daughters and carnotite ore dust,
   c. diesel engine exhaust alone,
   d. diesel exhaust fumes, radon daughters and ore dust,
   e. carnotite ore dust.

Concentrations of radon and daughters, composition of ore dust and concentrations of other toxic agents in the diesel oil exhaust fumes were carefully controlled. After being exposed to predetermined cumulative dosage levels comparable to the WLM exposures of high-level exposed miners, the animals were maintained for life for the observation of pathological changes, particularly lung cancer.

2. To test specifically the possible synergistic effects of radon and daughters and cigarette smoking, studies were initiated on three groups of 18 dogs as described below:

   a. dogs exposed to radon and daughters on carnotite ore--exposure for four hours per day, 7 days per week at concentrations of 15 nanocuries per liter; with sham smoking;

   b. dogs exposed to radon plus daughters on ore as in (a), plus two working shifts of smoking (total of 10 cigarettes per day with resting on alternate hours);

Regular clinical, radiological and radiographic examinations will be made during and after the smoking and radon exposures. The dogs, except for one in each

group which will be sacrificed for early histopathological studies, will be held for their remaining life span with observations of pulmonary changes and lung cancer as the end point.

MAJOR FINDINGS: Phase II of the hamster studies has been in progress for nearly 17 months, consisting of six groups of male hamsters that are simultaneously receiving daily 6-hour inhalation exposures to laboratory air (controls), 1200 WL of radon daughters, 1200 WL radon daughters with 15 mg/m^3 uranium ore dust alone, diesel engine exhaust and diesel engine exhaust with 1200 WL radon daughters and uranium ore dust. Hamsters sacrificed to date have shown respiratory tract lesions covering a spectrum ranging from normal lungs in control hamsters to metaplasia of bronchial and bronchiolar epithelium and adenomatous hyperplasia of bronchioloalveolar epithelium (cuboidal metaplasia or bronchiolization) in hamsters in the experimental groups. The most frequent finding was that of focal accumulations of particulate-laden macrophages adjacent to small bronchioles and vessels; where such particle deposits were heaviest, they were frequently accompanied by septal cell hyperplasia. This macrophage accumulation appeared within a few months and was found in almost all of the hamsters exposed to diesel engine exhaust alone or with radon daughters and uranium ore dust. Similar accumulation of macrophages was found in about 50% of the hamsters that inhaled uranium ore alone or with 1200 WL radon daughters after more than five months of exposures. Cases of hyperplasia and metaplasia began to appear after 5-6 months of exposures, increasing sharply thereafter; adenomatous lesions began to appear after six months. Mortality, body weight and hematology of experimental groups to date show no significant difference from controls.

Sixty beagle dogs are continuing to receive daily inhalation exposures to cigarette smoking and/or 600 WL of radon daughters with uranium ore dust. These animals have received 30 months of exposures to date. Determinations of carboxyhemoglobin levels in all dogs are being correlated with individual smoking patterns. Radiographs show no changes in control animals, but one dog exposed to radon daughters with ore has shown a diffuse lesion in the left lung suggestive of pneumonia. No significant differences from controls have appeared in respiratory rates and volumes or body weights; total white blood cell counts and absolute segmented neutrophil levels have risen significantly higher in the group receiving cigarette smoking plus radon daughters with ore during the last 12 months. Lung washings of the dogs in this group and in the group receiving radon daughters with ore show large macrophages with large numbers of neutrophils and occasional lymphocytes. Pathological findings of the lungs of four dogs sacrificed six months after beginning exposures include perivascular and peribronchiolar accumulations of macrophages in the dog inhaling cigarette smoke, with early epithelial changes; hyperplastic and metaplastic bronchiolar epithelium, fibrosis, peribronchiolitis and perivasculitis in the dog inhaling radon daughters with ore; and similar but more extensive changes including vesicular emphysema, focal areas of fibrosis and scattered and focal squamous metaplastic lesions with morphologic atypia in the dog inhaling cigarette smoke and 600 WL radon daughters with ore. Histopathologic examination of tissues from a dog obtained after 21 months of exposures also showed diffuse distributions and focal accumulations of macrophages laden with particulates adjacent to small bronchioles and vessels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These experiments are designed to determine, in both a short-lived animal, the hamster (life-span 2-3 years), and a long-lived species, the dog (life-span 12-13 years), the possible synergistic effects of chemical agents--dust, cigarette smoking and diesel exhaust fumes--operating singly and in concert with exposure to radon and daughters upon the production of lung cancer in animals. To the extent possible in the laboratory, the exposure conditions are designed to simulate those to which uranium miners have been exposed, and in whom an increased incidence of lung cancer is now appearing.

PROPOSED COURSE: The hamsters exposed to 1200 WL radon daughters and 1200 WL radon daughters plus uranium ore dust will reach their life spans and results will be analyzed during the next contract year. The exposures to diesel exhaust and radon daughters will continue. The dog exposures to cigarette smoke, ore dust and radon daughters will also continue.

BATTELLE PACIFIC NORTHWEST LABORATORIES, RICHLAND, WASHINGTON (NIH-72-2043)

TITLE: "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): L. Fishbein, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $121,496.00

PROJECT DESCRIPTION

OBJECTIVES: To determine by both radio-tracers and techniques, such as high resolution pressurized liquid chromatography, GLC-mass spectroscopy and electron spin resonance spectroscopy, the distribution of heavy metals (e.g., Tl, Sb, Sn, Mn, Ag, W, Ni, Co, Cd, Zn and Cr), their complexes of environmental significance on soil particles, microorganisms and solution and in representative grass and broadleafed plants grown on treated soil. The determination of the rate and extent of uptake and translocation of the metallic compounds within the plants as well as the nature of the bond types and chemical forms of the metabolites in microbial and plant tissues will also be studied.

METHODS EMPLOYED: Various conventional chromatographic (TLC, column, GLC, and liquid) and spectroscopic (u.v., visible, I.R., N.M.R. and mass) and radiochemical techniques.

MAJOR FINDINGS: To early to have any results.

DUKE UNIVERSITY, DURHAM, NORTH CAROLINA (NIH-NIEHS-72-2094)

TITLE: "A Study of Microwave-Neuron Interactions"

CONTRACTOR'S PROJECT DIRECTOR: William T. Joines, Ph.D.

PROJECT OFFICER (NIEHS): Robert H. Wyatt, Ph.D.

DATE CONTRACT INITIATED: June 1, 1972

CURRENT ANNUAL LEVEL: $9,947.00

PROJECT DESCRIPTION

OBJECTIVES: The contract objective is to determine how microwave energy within the 0.1 to 3.0 GHz frequency range interacts with the human nervous system.

MAJOR FINDINGS: Theoretical results, using a model of the skull, show that there are a number of resonant frequencies where the microwave energy absorbed by the skull exceeds the energy incident upon the shadow cross-section. Of particular significance is a strong relative absorption peak of about 1.5 occurring at 2.45 GHz, which would indicate that the leakage from microwave ovens operating at this frequency may be a greater hazard to human health than is now being recognized.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential hazards associated with microwave radiation are a matter of current concern for NIEHS as a priority environmental hazard. The potential biological effects of microwaves form the basis for extant "safe levels." The indicated selective absorption of microwave energy by the skull, as well as the reported nervous system response to microwaves, are compelling reasons for a definitive study of microwave-neuron interactions.

PROPOSED COURSE: Various mechanisms for possible interaction between microwaves and isolated neurons will be investigated. Measurements will be made in an attempt to determine if there are certain changes in neuron behavior. Several electrical models of neurons which support an interaction with microwaves have been described in the literature. The merits of these models will be evaluated, and, with the results of this study, an attempt to arrive at the mechanism most likely to account for microwave-neuron interactions will be made. Initial experimental studies will use the abdominal ganglion of aplysia (a small marine animal), but as the studies progress, the nerve cells of other animals will be used also. No human subjects will be used. It will be important to know how much energy is absorbed by a neuron, and careful developmental work will be required to arrive at the most appropriate experimental arrangement. An initial arrangement has been proposed.

GEORGIA INSTITUTE OF TECHNOLOGY, ATLANTA, GEORGIA (NIH-71-2374)

TITLE: "Study of Microwave Dosimetry"

CONTRACTOR'S PROJECT DIRECTOR: Harold Bassett

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D.

DATE CONTRACT INITIATED: June 25, 1971

CURRENT ANNUAL LEVEL: $33,155.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of the contract is to develop techniques and detectors which can be used to measure the energy absorbed in a biological specimen placed either in the near or far microwave fields of frequencies ranging from 1 to 10 GHz.

MAJOR FINDINGS: Georgia Institute of Technology has developed the technology to fabricate microwave detectors using a pyroelectric crystal (triglycine sulfate). A probe has been built using a 0.5 cm x 0.5 cm slab of the crystal with excellent response at the 1 mW/cm^2 power level. The present probe must be placed in a modulated field, or itself be modulated, due to the fact that the probe measures a rate of change in temperature rather than an absolute temperature.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Instrumentation for the measurement of microwave energy is very limited. At present, there are no broad band detectors which are adequate for measuring energy absorption in biological material. With further development, this probe has the potential of becoming an accurate, sensitive detector for microwave fields of 1 - 10 GHz frequencies.

PROPOSED COURSE: The contractor has written a renewal proposal for another year of support at approximately the same level. The probe will be further developed in the following manner: (1) the probe will be miniaturized to the extent possible, (2) the absorption mechanism of the crystal will be studied so as to obtain optimum sensitivity and (3) the probe will be adapted so that it can measure continuous wave microwave fields.

PUBLICATIONS

Huddleston, G. K. and Bassett, H. L.: Studies of microwave dosimetry. Presented at Symposium on Electromagnetic Hazards, Pollution and Environmental Quality, Purdue University, Lafayette, Indiana, May 8-9, 1972.

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, ALBUQUERQUE, NEW MEXICO
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Respiratory Tract Deposition Models"

CONTRACTOR'S PROJECT DIRECTOR: Otto G. Raabe, Ph.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: November 11, 1971

CURRENT ANNUAL LEVEL: $103,119

PROJECT DESCRIPTION

OBJECTIVES: The major objectives of this research are:

(1) Construction of physical models of the respiratory tracts of man and several animal species;

(2) Measurement of the deposition of aerosols as small as 0.01 micron diameter in the models;

(3) Determination of deposition of selected particle sizes in animals and in man if practical;

(4) Development of a theoretical model of deposition in the lung based on the above results;

(5) Field evaluation of the physical-theoretical model.

MAJOR FINDINGS: No major results are available at this time. Construction of physical models and production of nearly monodisperse aerosols are proceeding satisfactorily.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A respiratory tract deposition model is needed to support research on the effects of exposure to chemical, biological and physical aerosols. Such a model would aid in establishing quantitative dose-response relationships in planned laboratory experiments and would effect more meaningful extrapolations to human exposure situations.

MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASSACHUSETTS (NIH-69-77)

TITLE: "Beryllium Case Registry"

CONTRACTOR'S PROJECT DIRECTOR: H. Kazemi, M.D., Chief, Pulmonary Unit

PROJECT OFFICER (NIEHS): Douglas H. K. Lee, M.D., Ph.D.

DATE CONTRACT INITIATED: December 19, 1968

CURRENT ANNUAL LEVEL: $36,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to maintain the Beryllium Case Registry originally set up in 1952 by Dr. Harriet Hardy, providing for: (a) periodic appeal to physicians to report all suspected cases of beryllium poisoning; (b) examination of reports by an expert panel to decide admission to the Registry: (c) acquisition of additional evidence of diagnostic significance; (d) diagnostic assistance to the reporting physician; (e) periodic follow-up on progress of cases.

The importance of maintaining the Registry as an information source lies in: (a) inadequate knowledge of late disease manifestations; (b) changing patterns of exposure to beryllium; and (c) uncertainties about the basic patho-physiological processes.

MAJOR ACCOMPLISHMENTS: The Registry has 817 cases listed, of whom 540 are known or believed to be alive. Some difficulties in completing the 1969 follow-up have been resolved. A revalidation of old entries has been completed, and stricter rules are applied to the admission of new cases. There were 11 new cases added in 1971 and 27 specimens were examined for Be. All radiographs have been re-examined on a uniform basis. Sixty physicians attended a meeting on BCR and berylliosis. Members of the BCR team assisted in a study of an apparent exposure incident in a Be plant.

PROPOSED COURSE: Continue operation of BCR. Start another triennial follow-up of old cases.

MIDWEST RESEARCH LABORATORIES, KANSAS CITY, MISSOURI (NIEHS-72-2084)

TITLE: "Long-Range Toxicologic Effect of Metal-Containing Dithiocarbamate Fungicides"

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): L. Fishbein, Ph.D.

DATE CONTRACT INITIATED: February 1, 1972

CURRENT ANNUAL LEVEL: $98,859.00

PROJECT DESCRIPTION

OBJECTIVES: (1) To establish the chemical stability of metal-containing dithiocarbamate fungicides such as Ferbam, Ziram, Nabam, Sineb and Maneb, and to determine their degradation products in formulations upon storage over a 2-month period and in formulations of actual use on moist grain; (2) to establish the stability of these compounds incorporated in the animal feed for use in the animal studies; (3) to develop analytical methods and obtain a pesticide profile in animal feed for DDT, DDE, dieldrin, endrin, aldrin and heptachlor epoxide; (4) to analyze the selected fungicides and animal feeds for trace mineral content (e.g., Co, Hg, Ni, Pb, Se, Tl and other metals of interest); (5) to analyze the bedding material used in the animal studies for the presence of phenols; (6) to study and complete the acute and subacute toxicities of 2 selected major degradation products or the parent dithiocarbamate fungicides in rats; (7) to study and complete the metabolism of one selected compound in rats; (8) to initiate teratogenic studies of one selected compound in rats and mice; and (9) to initiate the chronic toxicity of 2 selected compounds in rats.

METHODS EMPLOYED: Various conventional chromatographic procedures, u.v., visible I.R. and N.M.R. spectroscopy and histological techniques.

MAJOR FINDINGS: It is too early to report any findings for this study.

North Carolina State University, Raleigh, N. C.
(PH-43-68-1375)

TITLE: "Role of Fungi in the Production of Toxins in Cigarette Smoke"

CONTRACTOR'S PROJECT DIRECTOR: G. B. Lucas, Ph.D.

PROJECT OFFICER (NIEHS): Robert G. Owens, Ph.D.

ALTERNATE PROJECT OFFICER (NIEHS): Ronald W. Pero, Ph.D.

COLLABORATORS (NIEHS): R. G. Owens, Ph.D. and Ronald W. Pero, Ph.D.

DATE CONTRACT INITIATED: July 1, 1968

CURRENT ANNUAL LEVEL: $70,000

PROJECT DESCRIPTION

OBJECTIVIES: The objective of this project is to determine the kinds and amounts of toxins contributed by molds to the solids of cigarette smoke. More immediate objectives dealing with specific aspects of the overall goals consist of acquisition of reference standards for the major toxins produced by the molds associated with tobacco. In most cases it is necessary to isolate these from the molds of interest, since few of the toxins are available from commerical or other sources. It also involves development of methods for isolation and quantitative assay of the toxins. As methodology is developed, the toxin contents of moldy tobacco and smoke condensates are determined. The influence of environmental factors and strain characteristics on toxin production by the various organisms will also be determined.

METHODS EMPLOYED: Since the studies spanned a number of aspects of mycotoxicology and several toxins, requiring a variety of biological and chemical procedures, the methods employed can be indicated best relative to "Major Findings" in each study area.

MAJOR FINDINGS: Toxic metabolites of Alternaria tenuis. Tests were carried out to determine if alternariol and/or alternariol monomethyl ether (AME) is produced by A. tenuis in cured tobacco and if the compounds occur in appreciable quantities in tobacco in the marketplace. Extraction and analytical procedures were those worked out previously at NIEHS, with lower detection limits of 0.1 ug.

Shredded flue cured tobacco inoculated with the fungus and incubated under conditions favorable for fungal growth yielded small quantities of alternariol (2.1 to 2.3 ppm) and AME (1.7 to 1.8 ppm). None of some 40 tobacco samples collected from the marketplace yielded detectable quantites of either toxin. It is concluded that alternariol and AME are not produced in large amounts in cured tobacco even under the most favorable conditions. Thus, they are probably inconsequential in smoke solids of marketed flue-cured tobacco.

Effects of nitrogen and carbon sources on production of alternariol, AME and altenuene by A. tenuis. Six carbon and two nitrogen sources, each at four concentrations, were compared for quantitative effects on alternariol, AME and altenuene production in defined media. The compounds in extracts of the cultures were quantitated by thin layer chromatography. Maximum production of each toxin occurred in media with high C:N ratios. The most favorable carbon sources were sucrose and xylose. The most effecient nitrogen source was asparagine. However, the carbon source had a more pronounced effect on toxin production than did the nitrogen source.

Relative toxin production by different strains of A. tenuis. Ten different isolates of A. tenuis were tested on the same culture medium for production of alternariol, AME and altenuene. The toxins were quantitated by thin layer chromatography and extracts of the cultures were tested for toxicity to Bacillus megaterium and B. mycoides by the filter paper disc bioassay technique. The isolates differed both in toxicity to the test bacteria and in the amounts of the toxins produced. No correlation was seen between inhibition of test organisms and production of the known toxins. It was concluded that some as yet unidentified toxins contribute to the antibiotic spectrum of Alternaria strains and that the various strains may differ substantially in toxigenic properties with respect both to known and unknown toxins.

Biosynthesis of ^{14}C-labeled Alternaria toxins. Several experiments by the group at N.C.S.U. revealed that the most efficient medium for biosynthesizing ^{14}C-labeled alternariol (and analogs) was one which contained asparagine and xylose as nitrogen and carbon sources and the vitamins riboflavin, thiamine, biotin, pyridoxine and niacin. The most nearly satisfactory labeling of toxins has been obtained with a mixture of ^{14}C-labeled xylose, asparagine and sucrose. Alternariol and AME with specific activities of 15 and 10 μC/mM, respectively, were obtained. This represents an increase of 10 to 20-fold in specific activity over that obtained by any other culture conditions tried. Efforts are now being made to effect a further 10-fold increase in specific activity by increasing the amount of labeled substrate added and introducing the labeled material several times, instead of once, during the incubation period. The goal is to obtain toxins with specific activity of the order of 100-150 μC/mM, which will be suitable for a variety of metabolic studies and for tissue distribution and metabolite determinations.

Gas chromatographic analysis of penicillic acid. Penicillic acid is a carcinogenic metabolite of at least two known fungi, Penicillium cyclopium and Aspergillus ochraceus. We have found it to be produced in cured tobacco, as well as other substrates, in substantial quantities by both organisms. Both of these fungi also produce another well-known carcinogen patulin. Thus, studies were undertaken to find suitable analytical procedures for both compounds simultaneously.

The two compounds were separated and quantitated satisfactorily by gas chromatography. Trimethylsilyl derivatives chromatographed with good peak characteristics on 3% OV-17, 3% Dexsil 300 and 3% OV-25 using three different temperature programs. Limits of detection varied between columns, with 3% Dexsil 300 being somewhat superior to the others. The lower detection limit with this column was 0.025 μg.

In practical trials with extracts of moldy rice and corn, penicillic acid was resolved satisfactorily from interfering substances with either 3% OV-17 or 3% Dexsil 300. However, patulin was resolved from other components only on 3% OV-17. Thus, OV-17 was considered the column of choice when working with extracts of farm commodities.

In contrast to purified samples, the practical detection limit for penicillic acid in crude extracts was about 1 µg/100 µg extract. However, with TLC cleanup of extracts much smaller amounts of the toxin can be quantitated, since recovery from the cleanup procedure is essentially quantitative.

Analysis of tobacco and smoke condensates for penicillic acid. Tobacco and smoke condensates were analyzed by gas chromatography for penicillic acid. Inoculated tobacco and naturally moldy samples from storage were used. Tobacco inoculated with Aspergillus ochraceus yielded up to 1800 µg/100 g. tobacco. That inoculated with Penicillim cyclopium yielded somewhat less (320 µg/100 g.). Commerical moldy samples yielded 11 to 23 µg/100 g. (mixed mold populations).

Analysis of smoke condensates from commercial unfiltered cigarettes spiked with penicillic acid indicated that approximately 3% of the toxin was transported unchanged in the smoke.

These studies show that penicillic acid is produced in cured tobacco stored under conditions where molds, especially A. ochraceus and P. cyclopium, can develop and that the compound can be transported in the smoke stream of cigarettes. This combination of facts logically suggests that penicillic acid could be a potential contributor to the long term hazard of cigarette smoking.

A new toxin from Aspergillus achraceus and other fungi. Of 12 fungal species isolated from tobacco, three (Aspergillus ochraceus, A. clavatus and Epicoccum nigrum) were found to produce a common metabolite toxic to Bacillus megaterium. The metabolite was produced in tobacco as well as in artificial medium.

Partial purification of the toxin was achieved by column chromatography. Both column and thin layer chromatography and a number of physical characteristics indicated the substance is unlike ochratoxins A and B, patulin, or penicillic acid. It is stable in weak acid and base, heat stable, and dialyzable. It fluoresces green to green-brown under UV. Further efforts are being made to identify the compound and to determine its mammalian toxicity, if any.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Some forty million Americans are smokers and may inhale products of toxigenic molds associated with cigarette tobacco along with pyrolysis products of tobacco per se. Some fungi associated with stored tobacco have been shown to produce carcinogenic compounds in peanuts, grains and other agricultural commodities. However, little work has been done on the ability of fungi to produce volatile toxins in tobacco. This study was conducted to determine if fungi do constitute a hazard to smokers beyond that already recognized

to be due to tobacco pyrolysis products themselves, and, if so, to determine the magnitude of the problem.

PROPOSED COURSE: This contract was terminated December 31, 1971.

PUBLICATIONS

Pero, R.W., Harvan, D., Snow, J.P., and Owens, R.G. A gas chromatographic method for the mycotoxin penicillic acid. Jour. Chromatography 65: 501-506, 1972.

OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE
(Contract No. to be assigned)

PROJECT TITLE: "Environmental Mutagen Information Center"

CONTRACTOR'S PROJECT DIRECTOR: H. V. Malling, Ph.D.

PROJECT OFFICER (NIEHS): W. G. Flamm, Ph.D.

DATE CONTRACT INITIATED: June 1, 1972

CURRENT ANNUAL LEVEL: $102,000

PROJECT DESCRIPTION

OBJECTIVES: The primary goal of the Environmental Mutagen Information Center is the accumulation, storage, retrieval and dissemination of all pertinent information concerning chemical agents in the environment that have been tested for mutagenicity.

METHODS EMPLOYED: Papers are abstracted and keyworded, bibliographic data is entered into computers and information from other computerized abstracting services is utilized by the Center. Search profiles against five screening services (MEDLAR, University of Georgia Computer Center, Institute for Scientific Information, Nuclear Science Abstracts and Biosis) are carried out and provide much of the basic data.

MAJOR FINDINGS (progress): The information system has been converted to the generalized Bibliographic Format of the Environmental Information System at Oak Ridge National Laboratory. This will permit the location of computer terminals at NIEHS and other Government Agencies. Over 6,000 items are now cited in computer banks and in data storage files. The total number of documents accumulated for 1972 was 2,200 of which 1,850 were included in an EMS-Newsletter along with a bibliography, author index and a permuted title index. Most of the 1971 literature on chemical mutagenesis has been collected and will be ready for publication in November of 1972.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recognition by public health officials and the scientific community that chemical agents may constitute a greater threat to man's genetic health than radiation has lead to the organization and development of the Environmental Mutagen Information Center. The Center provides a basic and important function pursuant to the development of a better understanding of mutagenesis.

PROPOSED COURSE: Continuing efforts to accumulate and store information pertinent to chemical mutagens and mutagenesis are planned. Tabular abstracting and keywording of abstracts will be initiated including the development of a chemical glossary which will provide the capability for performing structural searches. Comprehensive reviews will be prepared dealing with special areas of environmental mutagenesis.

PUBLICATIONS

Malling, H. V., Wassom, V. S., Von Halle, E. S., Nawar, M. M. and Medill-Brown, M.: A survey of the 1970 literature on chemical mutagenesis. EMS Newsletter No. 5 (Suppl. 1) 1972. (In Press).

Nawar, M. M.: Literature citations on the mutagenicity of some food additives. EMIC Pamphlet No. 7: 34-53, 1972.

Medill-Brown, M.: Literature citations on the teratogenicity of some food additives. EMIC Pamphlet No. 7: 1-33, 1972.

Generoso, W. M. and Von Halle, E. S.: Literature citations on the effect of chemicals on gonads. EMS Newsletter No. 5 (Suppl. 2) 1972. (In Press).

THE REGENTS OF THE UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN (NIH-71-2373)

TITLE: "Study of Microwave Dosimetry"

CONTRACTOR'S PROJECT DIRECTOR: Ralph E. Hiatt

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D.

DATE CONTRACT INITIATED: June 29, 1971

CURRENT ANNUAL LEVEL: $51,525.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of the contract is to develop techniques and detectors which can be used to measure the energy absorbed in a biological specimen placed in either the near or far microwave fields of frequencies ranging from 1 to 10 GHz.

MAJOR FINDINGS: Small magnetic field probes have been built and calibrated in free space. These probes respond to the intensity of the magnetic field of the electromagnetic wave. Problems have been encountered in calibrating the probes in high dielectric constant materials and at high frequencies. These difficulties are due to the moding of the electromagnetic field in these materials. Therefore, the field in which the probe is placed is not well known. A technique for extrapolating the results to higher dielectric constant materials and higher frequencies has been theorized and will be applied in future work.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This probe will be useful in our research into the bioeffects of microwave radiation. The probe is small and can be used to measure free field power densities without disturbing the field and can also be implanted in biological specimens. The broad band capability of the probe will be needed in our frequency dependent research.

PROPOSED COURSE: The contractor plans to verify the technique for calibrating the probe in the high dielectric material and at high frequencies. A probe will be calibrated for free field conditions and in material of various dielectric constants. The contract will be discontinued at the contract expiration date, June 28, 1972.

SOUTHWEST RESEARCH INSTITUTE, SAN ANTONIO, TEXAS (NIH-72-2035)

TITLE: "The Interaction of Specific Air Pollutants with Lung Lipids"

CONTRACTOR'S PROJECT DIRECTOR: R. Bollinger, Ph.D.

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $161,577

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the effects and mechanisms of interaction of specified gaseous compounds with lung membrane lipids in vivo and in vitro; to clarify the chemistry and biochemistry of such interactions; to attempt to correlate the structural changes observed on a chemical level with any significant functional changes; and to examine possible reversal, protective or preventative approaches.

METHODS EMPLOYED: The basic test animal is the rabbit, in vitro systems include lung slices, lung mitochondria and cells from lung washes. Various conventional chromatographic procedures, NMR and IR spectroscopy and histological techniques are used. ESR is used to detect alterations in membrane components in situ.

MAJOR FINDINGS: Animals have been equilibrated on the standard diet and baselines have been established for most of the analytical subjects. The techniques to be used have been tested and standardized. Exposure Chambers have been constructed and installed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance cannot be evaluated until some experimental results have been obtained.

PROPOSED COURSE: To continue as outlined in the contract.

UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Uranium Mining - Health Hazards Associated with Radon Daughters"

CONTRACTOR'S PROJECT DIRECTOR: William F. Bale, M.D.

PROJECT OFFICER(NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: June 30, 1968

CURRENT ANNUAL LEVEL: $119,000

PROJECT DESCRIPTION

OBJECTIVES: In five separate but closely interrelated projects, senior scientists have and/or are conducting the following studies:

Project A - Dr. Donald Morken has exposed dogs and rats to radon and radon daughters on room air nuclei. The magnitude and intensity of exposure versus early histopathological effects are important initially.

Project B-1 - Dr. John Hursh has studied the bone and tissue distribution of ^{210}Pb as well as excretion of ^{210}Pb and ^{210}Po using dogs exposed in Project A.

Project B-2 - Dr. Hursh has measured the transport time of ^{212}Pb from human lungs.

Project C - Dr. Thomas Mercer has characterized the radon daughter carrying aerosols used in the studies by Morken and Hursh.

Project D - Dr. R. W. Helmkamp and Dr. W. F. Bale have developed new techniques for the measurement of ^{210}Po and ^{210}Pb in urine.

MAJOR FINDINGS: Project A - Effects on Dogs of Radon Decay Products - A total of 40 dogs have been exposed to radon daughter levels from 250 - 10,000 WLM. Pathological findings on 18 dogs sacrificed prior to FY 1972 have been reported. Tissue sections for study are now available for all experimental dogs, excluding 3 controls and one dog exposed to 10,000 WLM to be sacrificed in FY 73. A report on the pathological findings will be available in early FY 73. Measurements of the radio-lead content of representative bone specimens from all experimental dogs are now underway. A special experiment was conducted to determine the doses to various regions of the respiratory system per working-level-month exposure.

Project B1- - Measurement of ^{210}Pb Effective Half-Life in Dogs - The major portion of this program, including the analysis of about 2000 urine and fecal samples for radio-lead, has been completed. A final report of this work will be available by July 1, 1972.

Project B-2 - The Transport of ^{212}Pb from the Lung in Man - The effective half life for the loss rate of ^{212}Pb from the human lung was measured to be 10 - 12 hours.

Project C - This project was completed during FY 71.

Project D - Methods for Improved Bioassay of ^{210}Pb Body Burdens by Determination of ^{210}Pb and ^{210}Po in Urine and Feces - Dr. Helmkamp and Dr. Bale have developed a method which appears to quantitatively recover ^{210}Po from two liter collections of urine. A specially designed ionization chamber counter is used to improve counting efficiency; in effect eliminating many of the preparation steps previously necessary. By the end of FY 72, reports are expected on ^{210}Po and ^{210}Bi excretion in the urine of dogs exposed to a radon atmosphere or injected with ^{210}Pb and on a study of chemical techniques for isolating ^{210}Po from urine.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is an integral part of research directed toward the resolution of the uranium miner-lung cancer problem. This project should yield useful information on induction of lung cancer and on heavy metal metabolism.

PROPOSED COURSE: Projects B-1, B-2, and C have been completed. All experimental work on projects A and D is to be completed during FY 1973. Work during FY 74 will involve analysis of final data and preparation of final reports.

PUBLICATIONS

Hursh, J. B. and Mercer, T. T.: Measurement of ^{212}Pb loss rate from human lungs. Journal of Applied Physiology. (In press).

Mercer, T. T.: The deposition of Unattached Decay Product Atoms in an Impaction Stage. Health Physics. 17:259 (1969).

Blair, H. A.: Dose-time relations for induction of lung cancer in uranium miners. Reprint from symposium on "Radiation-Induced Cancer." International Atomic Energy Agency, Vienna (1969).

UNIVERSITY OF TEXAS; AUSTIN, TEXAS (NIH-70-2288)

PROJECT TITLE: "Detection of Human Somatic Mutations"

CONTRACTOR'S PROJECT DIRECTOR: H. Eldon Sutton, Ph.D.

PROJECT OFFICER (NIEHS): W. G. Flamm, Ph.D.

DATE CONTRACT INITIATED: June 30, 1970

CURRENT ANNUAL LEVEL: $32,444.00

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this study is to design and develop methods for measuring the frequency of somatic mutations at several genetic loci in humans. Peripheral blood cells are the tissue studied because production of these from a stemline leads to the accumulation of many more mutants than occur in tissues with slower turnover of cells. We initially proposed to investigate two systems, one being glucose-6-phosphate dehydrogenase (G6PD), the other being the red cell hemoglobin. In the case of G6PD, many variants are known in man, some of which have broadened substrate specificities. This appeared to offer opportunities for detecting variant forms. In the case of the hemoglobins, there exists a codominant gene for high fetal hemoglobin. In theory, it should be possible to detect red cells that contain high levels of fetal hemoglobin by the staining procedure of Betke.

METHODS EMPLOYED: Histochemical techniques are used to quantitatively assess the presence of rare mutant varients occurring at the glucose-6-phosphate dehydrogenase (G6PD) locus on the X-chromosome. This involves the employment of a substrate (2-deoxyglucose-6-phosphate) and co-factor (DPN) which can not be metabolized or utilized by the wild-type form of G6PD. The mutant cell, with its altered G6PD enzyme, will oxidize 2-deoxyglucose (to 2-deoxygluconic acid) and reduce DPN to DPNH. By adding phenozinemethosulfate and a tetrazolium salt, DPNH reacts to form a dark blue, insoluble formazan which is microscopically visible. In the absence of added substrate, but in the presence of DPN, no detectable production of formazan occurs. An effort is also being made to adapt the Betke method of staining fetal hemoglobin in red cells to a suspension method.

MAJOR FINDINGS: Many of the variables effecting the ability of peripheral blood cells to metabolize 2-deoxyglucose phosphate have been identified. However, the lack of association between variant frequency at the G6PD locus and the age of the individual under test raises serious doubts as to whether mutations are primarily responsible for the presence of variant cells. Indeed, it is now recognized that certain oxidizing agents can convert normal G6PD into a form having properties of the variant cells. It might be that variant cells simply represent instances where G6PD has undergone denaturation *in situ* leading to a loss of substrate specificity. If, however, this event were only a minor contributor to the development of the variants, it would not negate the applicability of the G6PD system for the detection of mutants.

This is the central question insofar as studies of the G6PD locus is concerned, and efforts are continuing to clone cells to establish the heritability or lack of and thus to arrive at a satisfactory answer. The recent observation that the frequency of variants in different persons under identical laboratory situations is exactly the same whether galactose-6-phosphate or 2-deoxyglucose-6-phosphate is used as substrate offers hope that the system might actually be functioning as intended.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of systems which can be used to monitor the exposure of individuals to mutagenic events is of considerable bio-medical importance. Such exposures undoubtedly occur as a consequence of certain occupations and as a result of the ubiquity of mutagenic agents in the environment. A number of conferences on mutation testing have confirmed the earlier opinions that ultimately the monitoring of mutation in human populations should be through measure of somatic mutations. Even those who are convinced of the importance of monitoring germinal mutations agree that identification of specific environmental agents and assessment of risk to specific exposure groups is likely to be possible only through detection of somatic mutations.

PROPOSED COURSE: Because we are not satisfied that the laboratory methodology justifies an extensive testing of human subjects, we would like to continue to explore properties of the variant G6PD reaction with the likelihood that studies of this enzyme will be terminated as soon as they are carried to point where publication of results is possible. Among the specific approaches to be used is the study of lymphoblastoid cell lines. This was mentioned as a possible objective last year, and we have now decided that this should be done very soon. With the use of such cultures, it may be possible to demonstrate that at least some of the variants are true mutations. This would be an important observation even though the proportion of variant cells due to mutation may be low and not really ascertainable. We expect to devote a very much greater portion of time to the high fetal variant during the coming year. Since the frequency of variant cells is so low in this system, it will be necessary to devise methods of counting which do not depend on counting individually the negative cells as well as the positive cells. The use of red cells has the disadvantage that there is no potential for demonstrating these variants to be heritable. Indirectly, one might be able to show an association with exposure to known mutagens, however. A systematic survey of other enzyme systems will be made during the year to search for other candidates. We will look especially at enzymes where variation is known to exist in affinity for cofactors or in susceptability to inhibition. For example, human glutathione reductase exists in several variants, each with different affinities for FAD. One variant has been identified that has an exceedingly low affinity for FAD. Another example, human carbonic anhydrase is a zinc-requiring enzyme. A rare varient exists which has several-fold greater activity at zinc levels ordinarily used in testing. Whether this is due to higher zinc affinity or simply higher activity per molecular complex is not known. Nevertheless, the possibility exists that under conditions of zinc restriction only those high activity cells would react positively.

PUBLICATIONS

Sutton, H. E.: The problem of surveillance. Teratology. 4: 103-107, 1971.

Sutton, H. E.: Prospects of monitoring environmental mutagenesis through somatic mutations. In Hook, E. B. (Ed.): Monitoring Birth Defects and Environment. Academic Press, New York, 1971, pp. 233-235.

Sutton, H. E.: Some new approaches in monitoring human populations for mutagenesis. Environmental Mutagen Society Newsletter. (In Press).

Sutton, H. E.: Monitoring somatic mutations in human populations. In Sutton, H. E. and Harris, M. I. (Eds.): Mutagenic Effects of Environmental Contaminants. Academic Press, New York, pp. 121-127.

U.S. FORESTRY SERVICE, RESEARCH TRIANGLE PARK, NORTH CAROLINA (NIEHS-72-2206)

TITLE: "Influence of Microorganisms and Other Soil Factors on Degradation of Certain Insecticides"

CONTRACTOR'S PROJECT DIRECTOR: Felton L. Hastings, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

ALTERNATE PROJECT OFFICER (NIEHS): Lawrence Fishbein, Ph.D.

DATE CONTRACT INITIATED: August 24, 1971

CURRENT ANNUAL LEVEL: $30,750.00

PROJECT DESCRIPTION

OBJECTIVES: To study the influence of microorganisms and other soil factors on degradation of certain insecticides; the insecticides of particular interest being Mirex, Kepone, DDVP, Disulfoton, Temik and Dursban.

METHODS EMPLOYED: (1) Develop adequate analytical procedures. (2) Determine the persistence of the insecticide. (3) Study the influence of the insecticide on soil microorganisms. (4) Study the metabolism of the insecticide by various microorganisms.

MAJOR FINDINGS: (Note: Studies to date have concentrated on Mirex.) It has been determined that the procedures previously recommended for analysis of Mirex in crops are adequate for analysis of Mirex in soils. The above procedures have been used to study the metabolism of Mirex in soil and the persistence of Mirex in the ant-bait formulation. No evidence of Mirex degradation was detected in either sterile or non-sterile soils containing Mirex, 0.1, 0.5, 1.0 and 2.0 g/100g soil for periods of from 1 to 3 months. Approximately 93.2% of the Mirex applied to an area as ant-bait remains in the bait 2 months after application. Mirex incorporated into the soil at concentrations from 0.01 to 1.0 grams per 50g soil had no apparent effect on the microbiology of the soil. The ant-baits are rapidly colonized by soil microorganisms, primarily fungi, which rapidly break down the oil in the bait, thus rendering it non-attractive to the ants after a few days. However, neither these fungi nor several bacteria tested have been found to break down Mirex.

PROPOSED COURSE: Studies are currently underway on Mirex metabolism over periods of 3 to 6 months in several types of soils containing a variety of microorganisms under aerobic and microaerophillic conditions. Similar studies are being conducted with lake bottom sediments under microaerophillic and anaerobic conditions. In addition, similar studies are being initiated on other insecticides.

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH
Summary Statement

The Research Services Branch is responsible for planning, coordinating and providing support services for the National Institute of Environmental Health Sciences. These functions include: 1) research facilities planning, design and construction; 2) plant engineering, alteration, operation and maintenance; 3) environmental sanitation and engineering services; 4) sterile glassware and media preparation; 5) security; 6) safety; and 7) landscaping and grounds maintenance. The Branch also conducts research on physical factors in the environment and develops and maintains instrumentation.

A new office building to house the Office of the Director and his immediate staff is currently in design. Occupancy is scheduled for July, 1973. The building will be located at the northern end of the NIEHS Interim Site.

Apportionment of the planning funds for designing the first stage of the NIEHS Permanent Center has been requested by NIH. Assuming this request will be approved, an architectural/engineering firm should commence design this summer. Construction funds will be requested in the FY 74 NIH budget. The Program of Requirements for the program facilities and the support services and site development were completed in February, 1972 by the Office of Engineering Services, NIH. OES did an excellent job in delineating the requirements of NIEHS as stated in these documents.

A variable frequency microwave exposure system is being installed and will complement the existing 2450 MHz microwave system. The frequency of the new system can be varied from 1000 to 12,000 MHz, and the power level varied from 0.01 to 10 mW/cm^2. The interaction of microwaves with matter is frequency specific. The addition of this new system will give the Institute greater flexibility in exploring the biological effects of microwave exposures.

The development of new and improved dosimetry techniques for microwaves is continuing. Techniques under investigation include the use of thermocouples, thermisters, magnetic probes and pyroelectric crystals. A majority of this work is being accomplished under contract. A calibrated broad band probe of the electric-field type for making free space measurements is being purchased from the National Bureau of Standards. This probe is relatively large and cannot be used for making measurements in biological mediums.

The development of an analytical tool for defining and quantifying trace elements in tissue and/or liquids using X-ray emission spectrometry looks promising. It appears possible to detect certain elements in the parts per billion range without extensive sample preparation or counting time. Inhouse efforts will continue in this area.

An audiometric room is being installed in Building No. 14. This capability will permit exposures of animal and other living organism to defined sound energy levels for study of auditory and/or non-auditory effects, singly or in combination with other agents.

Serial No.: NIEHS-RSB-001
1. Research Services Branch
2. Biophysics and Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Respiratory Tract Dose Model for Uranium Miners"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATOR: D. I. McRee, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to develop a respiratory tract dose model that will provide reliable estimates of the dose of radiation to the basal cells of the bronchial epithelium of the respiratory tract in the case of inhalation of alpha emitters.

METHODS EMPLOYED: Calculation of the dose to the basal cells in each anatomical region of the respiratory tract requires a detailed description of the exposure atmosphere, a detailed respiratory tract model, accurate techniques of deposition calculation and an accurate analysis of the effects of clearance.

MAJOR FINDINGS: Work on this project was re-directed during fiscal year 1972. A new contract was initiated to develop a respiratory tract deposition model (see Lovelace Foundation), and research was begun on novel techniques for determination of deposition in the respiratory tract (see NIEHS-RSB-017).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of an ideal respiratory tract dose model would require improved input information. This information would be crucial to the assessment of the risk associated with the possible inhalation of any toxic material. Such a model would have immediate applicability to the studies of the health hazards of uranium mining.

PROPOSED COURSE: Experimental measurement of the deposition of ions and particulates in models of the tracheobronchial tree is one example of information

needed to develop a comprehensive deposition theory (see NIEHS-RSB-007 and Lovelace Foundation contract). The effects of the wall characteristics and eddy currents at bifurcations will be important considerations. Additional physiological and biological information is also needed. The respiratory tract dose model will be elaborated in accord with availability of basic input information.

PUBLICATIONS

Walsh, P. J.: Radiation dose to the respiratory tract of uranium miners - A review of the literature. Environmental Research 3:14-36, 1970.

Walsh, P. J. and McRee, D. I.: Depth-dose curves for alpha particles from area sources and point sources. Health Physics 20:352, 1971.

Gastineau, R. M. and Walsh, P. J.: Thickness of bronchial epithelium with relation to exposure to radon. Health Physics. (In press).

Serial No.: NIEHS-RSB-002
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Energy Loss Characteristics of Radiation in Matter"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATORS: D. I. McRee, Ph.D.; Frances Pendergrass

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.4
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to obtain more information on the main interactions of ionizing radiation with matter, such as ionization and excitation of atoms and molecules, and the more subtle interactions of radiation with molecules, such as the effect of the penetrating radiation upon molecular bonds. Such information will be basic to further analysis of the connections between physical energy loss and absorption processes and biological effects.

METHODS EMPLOYED: The measurement of the energy loss of radiation is accomplished by using a solid state radiation spectrometer. The complete spectrometer consists of a measurement chamber containing an alpha, beta or gamma radiation source, multiple samples of the material to be studied and a solid state radiation detector. The doses and dose rates of alpha, beta and gamma radiation can be varied at will by adjusting the source-absorber geometry; and the solid state spectrometer allows an accurate determination of the energy loss in the absorber at the same time. This provides an experimental check on theoretical dose estimation. Biological absorbers can be studied with this system.

MAJOR FINDINGS: A theoretical model has been developed which describes the effect of dose-rate and turnover time of cells on dose-response relationships. The model provides a basis for planning experimental work as outlined under the proposed course. If experimentally verified, the theory will allow extrap-

olation of dose-effect data to low doses; heretofore not possible.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Further information on the nature of the interactions of ionizing radiation with matter is necessary for more accurate dose calculation and for improving our understanding of the connections between damaging insults to atoms and molecules and subsequent biological effects.

PROPOSED COURSE: This project may be expanded to:

1. Study the energy loss characteristics of radiation in cell suspensions and cell cultures and develop quantitative dosimetric concepts which describe the results;

2. Deliver known doses of radiation to cells at various dose rates to determine dose-response relationships as a function of dose rate and turnover time of the cells;

3. Study the relative effectiveness of alpha, beta and gamma radiation for a given end point;

4. Use chemical or biological toxicants or protectors in conjunction with the radiation. Such studies could identify the most important molecular components in terms of biological response.

PUBLICATIONS

Walsh, P. J.: On the application of the uncertainty principle to stopping power theories. Health Physics 19:574, 1970.

Walsh, P. J.: Stopping power and range of alpha particles. Health Physics 19:312-316, 1970.

Walsh, P. J.: Possible effects of dose rate and turnover time of cells on dose response relationships: implications for radiation protection guidance. International Journal of Environmental Studies 2:189-193, 1971.

Walsh, P. J.: Energy loss of alpha particles in tissue equivalent plastic. Health Physics. (In press).

Serial No.: NIEHS-RSB-003
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Services Branch

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Microwave Absorption by Biological Material"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: D. I. McRee, Ph.D., P. J. Walsh, Ph.D.

OTHER INVESTIGATOR: Philip E. Hamrick, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine the absorption of microwave radiation by biological material, to investigate the contribution of cell structure to the absorption and to attempt to explain the mechanism of interaction between microwaves and the biological material.

METHODS EMPLOYED: DNA, RNA, protein, cell cultures and biologically simulated material (alcohol-water solutions) will be exposed to 2450 MHz microwave radiation. The amount of energy absorption will be measured, and the changes in the media will be evaluated.

MAJOR FINDINGS: Studies have been made which tend to suggest that non-thermal microwave fields at various frequencies interact with intracellular moieties to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified. The absorption of microwave energy by RNA, DNA, protein and complete cells appears to be frequency dependent. It is not known whether the absorption of 2450 MHz microwave radiation is different in the above media. With the establishment of our variable frequency system, absorption at other frequencies will also be determined. Mouse lymphoma cells were exposed to 2450 MHz radiation. The cells were exposed at a power level of 90 mW/cm^2. From model experiments, it was determined that an equilibrium temperature of 37°C. could be maintained at this power level. The cells were exposed for 1 hour, 2 hour and 4 hour durations with specimens taken at the end of each exposure period. Tests for mutagenic effects

at two particular loci within the cell were made. These tests showed no effects.

Most of the effort this past year was concentrated on evaluating possible techniques for accurately measuring the absorbed energy. Preliminary results indicate that thermistor probes are presently the best available technique for use with our exposure system. Other type probes are being investigated through two contracts at Georgia Institute of Technology and University of Michigan.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanisms of interaction of microwave radiation and biological material need to be specified before an accurate evaluation of effects can be obtained. Many investigators believe that all effects, so far observed, are thermal effects. However, some studies have shown effects which were not due to a rise in temperature. More basic information about the interaction mechanisms will assist in the interpretation of these non-thermal effects.

PROPOSED COURSE: Studies on the absorption of microwave radiation by simulated biological material will be performed in order to obtain a measure of the electrical properties of biological specimens. Cell components such as RNA, DNA and protein, as well as the cells themselves, will be exposed, and the energy absorption measured. By studying the absorption of particular molecules which make up the cell, an insight into the mechanism of interaction will be obtained.

Serial No.: NIEHS-RSB-005
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Microwave Exposure System and Microwave Dosimetry"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATORS: P. J. Walsh, Ph.D. and R. H. Wyatt, Ph.D.

COOPERATING UNIT: Georgia Institute of Technology, Engineering Experimental Station, Electronics Division

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to develop a microwave exposure system for biological material which has a well-defined, uniform field characteristic; to study the mechanisms of interaction of microwave radiation with matter; and to develop dosimetric relationships for energy absorption.

METHODS EMPLOYED: A thorough study of the research requirements on the biological effects of microwaves was completed. Based on these studies, a set of specifications was written for a microwave exposure system which would generate a uniform, low-level microwave field in a small laboratory space. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, chemical reactions, liquid crystals and photographic film are some of the technics which will be investigated.

MAJOR FINDINGS: A microwave exposure system with a frequency of 2450 MHz has been designed, built and the field calibrated. The microwave field at the specimen location was determined to be uniform within the design specifications with variation in power density as follows:

6 inch diameter area - ±5%
9 inch diameter area - ±10%
12 inch diameter area - ±15%

The system provides a power density range of 0.01 mW/cm^2 to 200 mW/cm^2.

Thermistors and thermocouples have been evaluated for interaction with the microwave field. The thermistor and thermocouples were placed in the field with only air as the surrounding medium. The thermistor was not effected whether the leads to the thermistor bead were or were not shielded and/or grounded. The thermocouple was very sensitive to microwave interference if the leads were not grounded. Even when grounded some instability was noted. In future absorption and biological effects research, the thermistor will be used to monitor temperature.

An analytical model for the power-time thresholds for lenticular damage has been developed. The absorption of energy in the eye results in a rise in temperature of the fluid in the lens. By determining the absorption and dissipation rate constants of the lens from existing data in the literature, an analytical model was developed for power-time thresholds for cataractogenesis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The well defined microwave field will allow the Institute to perform accurate quantitative studies of the effects of microwave radiation on biological systems at a frequency of 2450 MHz. Using the thermistor to measure the temperature profile of a specimen during irradiation enables us to duplicate this temperature in the controls using ordinary heating techniques. This procedure will enable us to separate thermal and non-thermal effects. The analytical model for threshold levels for the development of eye cataracts enables interested scientists to determine safe exposure levels and provides a technique for extension to all frequencies as more experimental data become available.

PROPOSED COURSE: Investigations to evaluate the thermistor and other techniques in measuring the absorption and interaction of microwave radiation with matter will be continued. Although the thermistor is not affected by the microwave field, it has not been fully determined if the presence of the thermistor in the biological material changes the absorption properties of the material. This possible effect will be evaluated along with other measuring techniques which do not necessitate the placing of metallic material into the specimen. One technique in the developmental stage consists of a closed small glass tube with a pressure transducer attached. This device will eliminate the metallic material in the specimen and, hopefully, will enable accurate measurement of changes in temperature. Determination of the electrical properties of biological material and the physical and chemical mechanisms of interaction between microwave radiation and biological material will also be studied.

PUBLICATIONS

McRee, D. I.: Thresholds for lenticular damage in the rabbit eye due to single exposure to CW microwave radiation: an analysis of the experimental information at a frequency of 2.45 GHz. Health Physics Journal, Vol. 21, Dec. 1971.

McRee, D. I., and Walsh, P. J.: Microwave exposure system for biological specimens. The Review of Scientific Instruments, Vol. 42, Number 12, Dec. 1971.

Serial No.: NIEHS-RSB-006
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Ultraviolet Dosimetry"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: P. J. Walsh, Ph.D.

COOPERATING UNIT: Chemical Mutagenesis Branch

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to measure the actual dose to the cells when exposed to ultraviolet radiation and to develop analytical dose-response relationships. A comparison of the relative biological effectiveness of the ultraviolet radiation with ionizing radiation will be studied.

METHODS EMPLOYED: An ultraviolet exposure unit using 2537 Angstrom wavelength source is being designed. The unit will be used to produce damage in cell cultures. A UV photomultiplier tube will be used to determine the amount of energy absorbed by the cells.

MAJOR FINDINGS: An ultraviolet source has been obtained and calibrated. An exposure chamber which allows a variation in dose rate has been built. Seventy-seven percent of the energy output of the source is at 2537 Angstrom wavelength The power density of the source ranges from 2 ergs/sec-mm^2 at 40 inches to 90 ergs/sec-mm^2 at 6 inches. In order to use the pulse height analyzer to measure the energy absorbed by the cells, the quantum density of the source must be drastically reduced. Spherical integrators have been designed, but have not as yet been built. These spherical integrators will reduce the quantum density and maintain the spectral integrity of the source.

Drs. Clive and Flamm of the Chemical Mutagenesis Branch have used the exposure system in an attempt to produce mutations. Dr. Phil Hamrick has used the system in an attempt to produce mutations in various strains of bacteria.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information on the amount of ultraviolet energy absorbed by a cell to a given amount of damage is important in studying the repair mechanisms and in comparing this damage to that caused by other types of radiation or agents. Knowledge of the actual dose to the cells can lead to analytical descriptions of the interaction which may be applied to other types of cell damage.

PROPOSED COURSE: The spherical integrators will be incorporated into the UV readout system so that not only the exposure to the cells but also the dose to or absorption of the cells can be measured using the photomultiplier-pulse height analyzer system.

The dose of ultraviolet radiation to cell suspensions and cultures will be measured, and theoretical dosimetric concepts which describe the phenomena will be made. A comparison of the relative biological effectiveness of the ultraviolet radiation to ionizing radiation will be performed in an attempt to develop broad dose-response relationships.

Serial No.: NIEHS-RSB-007
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Deposition of Aerosols in Tracheobronchial Tree Models"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Phillip J. Walsh, Ph.D.

OTHER INVESTIGATOR: D. I. McRee, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.15
Professional:	0.15
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to study the deposition of aerosols in respiratory tract models. Accurate measurements of the deposition will be made and an improved theory formulated for calculating the magnitude of deposition along the walls and at bifurcations.

PROPOSED COURSE: This project has been terminated. Work to be carried out on this project will be included in NIEHS-RSB-001 and in the Lovelace Foundation contract.

Serial No.: NIEHS-RSB-008
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Variable Frequency Microwave Exposure System"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: R. H. Wyatt, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.20
Professional:	0.20
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to design, build and calibrate a variable frequency exposure system for biological material.

METHODS EMPLOYED: Specifications were written for the desired system. A field uniformity at the specimen of 90 percent over a 4 inch diameter circle, a continuous frequency variation in the range of 1 to 10 GHz and a power level range at the specimen of 0.010 to 10 mW/cm^2 were specified.

MAJOR FINDINGS: Many of the biological effects of microwave radiation are frequency dependent. In terms of whole body irradiation, a frequency of 150-1,200 MHz affects the internal organs, whereas, a frequency of 10,000 MHz and above affects primarily the skin. Components of cells such as RNA, DNA and protein were found to have maximum absorption at different frequencies. Therefore, it is necessary to have the capability of varying frequency when investigating microwave effects on biological systems. The frequency range of 1 to 10 GHz was chosen because it is within this range of frequencies to which the general population is exposed.

All components for the system have been obtained except for the 1-2 GHz TWT-amplifier. However, the system is ready to operate at frequencies from 2-12 GHz. A broad band energy density probe has been ordered to calibrate the system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many biological effects of microwave radiation appear to be frequency specific. The variable frequency exposure system will provide the researchers of the Institute the capability of studying the effects of different frequencies on biological systems.

PROPOSED COURSE: The variable frequency system will be calibrated when the broad band probe is received. Two broad band probes being developed under contract will also be used to characterize the microwave field at the specimen location and to measure energy absorption in these specimens.

Serial No.: NIEHS-RSB-009
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Development of a Middle Ear Transducer"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. O. Cook

OTHER INVESTIGATORS: (Advisors*) L. Royster, Ph.D., North Carolina State University; G. Thomas, Ph.D., University of North Carolina at Chapel Hill, N. C.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.8
Professional:	0.6
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The immediate objective is to finish a workable prototype which will fit and remain in a normal (non-surgically-altered) mammalian ear at the level of the tympanic membrane. This problem has presented formidable obstacles because there is little to which it can be attached that would not permanently and deleteriously alter sensitive ear structures. In addition, the transducer must be coated with a very thin, tissue compatible, electrically insulative coating for the transducer. The first prototype is about 75% complete.

The transducer itself is a piezoelectric device which responds to an applied voltage with a movement which will activate ossicular chain or round window mechanically instead of acoustically. Accurately controlled movement in the Angstrom range is necessary because ossicular chain movement is less than 10^{-8} meters at normal sound pressure ranges.

The third step will be to take measurements by means of cochlear microphonic recordings of the ear's response to the stimulation.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The device as presently envisioned will be useful in delivering, in experimental work, a very precisely known signal to the ears. Extension and refinement of the concept could lead to a hearing aid without the low frequency noise problem

*Project is Ph.D. dissertation project of principal investigator

plagueing present hearing aids, which must generate sound acoustically into the ear canal.

Serial No.: NIEHS-RSB-010
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Adaptation of Zwicker loudness calculation scheme for annoyance evaluation"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. O. Cook

OTHER INVESTIGATORS: F. D. Hart, Ph.D., Director, Center for Acoustic Studies, North Carolina State University; S. E. Dunn, Ph.D., North Carolina State University

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is the redesign of the Zwicker Nomographs for calculations which measure the annoyance rather than the loudness of sounds.

METHODS EMPLOYED: Mathematical and graphical techniques, along with the curves for equal annoyance developed by K. D. Kryter, will be employed. Results will be compared with results of judgment tests previously published in the literature.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The method developed by Zwicker mimics the sound energy summation properties of the human auditory system by correctly accounting for spread of masking and critical band properties.

It was developed, however, for subjective loudness calculations, which seem to be the preferred subjective parameter in Europe, whereas the annoyance parameter has been more widely used in this country. The methods developed by Kryter for annoyance estimation give good results as long as the spectrum is a relatively broad band, but are subject to error where energy peaks are widely separated in the spectrum.

PROPOSED COURSE: To redesign the Zwicker Nomographs for calculations which measure the annoyance of sounds.

Serial No.: NIEHS-RSB-011
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Determination of Impedance of Middle Ear at two or three Atmospheres Pressure"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. Farmer, M.D., Duke University; G. Thomas, Ph.D., University of North Carolina at Chapel Hill, N. C.; Reginald O. Cook, Nat'l. Institute of Environmental Health Sciences

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0 (except for volunteer subjects)

PROJECT DESCRIPTION

OBJECTIVES: The objectives are to gain information relative to the change of impedance with increased pressure.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At present, only sketchy data exists on the sound transmissibility of the ear under several atmospherespressure. Knowledge of transmissibility characteristics will make possible a better understanding of the permanent and temporary threshold shifts noted in personnel using the Duke University hyper-baric chamber.

PROPOSED COURSE: Data was taken from three subjects. Our intention is to gather more data at greater pressures before publication.

Serial No.: NIEHS-RSB-012
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Investigation of the Effect of Low Level Incubator Type Noise in Combination with Various Drugs on Newborn Animals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. O. Cook and Stephen A. Falk, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.3
Professional:	0.8
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: To ascertain whether newborns are more susceptible to hearing damage than more mature animals, to test the synergism of ototoxic drugs with low level noise and to investigate whether 24 hour continuous noise exposure is more damaging than the 8 hour or less exposure from which nearly all conclusions have been drawn.

METHODS EMPLOYED: This project will allow us to make our first use of the new "sound proof" room slated to be installed in Bldg. 14 the week of April 15th. This facility will allow us to establish and precisely control sound fields for the first time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The above research forms, along with the noise-ototoxic drug contract expected to be let shortly, will enable us to carry out a balanced investigation of the basic mechanisms of hearing loss associated with environmental insults.

Serial No.: NIEHS-RSB-013
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The ototoxicity of methyl mercury"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: R. Klein, M.D.

COOPERATING UNITS: Pathology Branch

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to identify and evaluate the pathological effects of methyl mercury on the cochlea.

METHODS EMPLOYED: Two types of animals are used for exposure to methyl mercury--pregnant rats and young guinea pigs. Pregnant rats were treated with 15 daily subcutaneous doses of 2 mg Hg/kg body weight of methyl mercury hydroxide during gestational days 2 through 15. Hearing from the six-week old offspring of these pregnancies was assessed by the Preyer reflex, and the cochleas were studied histologically by surface preparations.

Young guinea pigs were treated with methyl mercury hydroxide (2 mg/kg/day, subcutaneously) for 1, 2, 4, 6 and 7 weeks. Hearing was assessed by the Preyer reflex and the cochleas were studied histologically by surface preparations.

MAJOR FINDINGS: Cochleas of the six-week old rats (whose mothers were treated with mercury) were found to be entirely normal on histological examination. Preyer reflexes also were normal.

However, the organ of Corti of the treated guinea pigs showed significant pathology. The inner hair cells and supporting cells (pillars, Deiters cells and cells of Hensen and Claudius) were normal. The outer hair cells, however, showed complete or partial collapse, loss of stereocilia and total degeneration in some instances. These changes were significant by Mann Whitney U Test at

the level of the cochlea at three and one-half turns from the base. The changes were present at two and one-half turns from the base, although they were not significant there. Pathology was found in the animals treated for 1, 2, 4, 6 and 7 weeks; and the degree of pathology did not vary with length of treatment beyond one week. The treated animals had normal Preyer reflexes, but this is not disturbing considering the redundancy of hair cell distribution in the cochlea, especially in the apical turns. As much as 20% of hair cells needs to be lost before hearing thresholds are raised.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The reports of human mercury poisoning include hearing loss of high frequency sensorineural type. Mercury, which causes a general sensory neuropathy, also affects the auditory nerve. However, these studies indicate that the hearing loss occurring in Minamata disease is also due to a primary cochlear lesion.

Cochlea lesions from methyl mercury are very unique. All the known ototoxic drugs (aminoglycoside antibiotics, potent diuretics) and noise cause cochlear pathology initially in the first turn from the base. As dosage is increased, or as treatment time is lengthened, pathological changes are observed progressively at more apical turns of the cochlea. Thus, methyl mercury represents a unique ototoxic agent; and it is hoped it can be used as a tool for revealing mechanisms of ototoxicity in general.

PROPOSED COURSE: Electronmicroscopic evaluation will be added to the pathological studies to find the initial locus of action of mercury ototoxicity. Studies using radioactive mercury will then be undertaken to determine cellular localization of the mercury to discover whether the pathology is secondary to cellular sensitivity or differential delivery or uptake by the cells of the apical turn.

Serial No.: NIEHS-RSB-014
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Combined Ototoxicity of Sodium Salicylate and Ethacrynic Acid"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: Dr. Jiri Prazma, Miss Ann Groce

COOPERATING UNITS: Division of Otolaryngology, University of North Carolina School of Medicine

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Salicylates, including aspirin and ethacrynic acid, are well recognized as having ototoxic effects on the cochlea. The objectives of this project are to determine their effects when administered simultaneously.

METHODS EMPLOYED: Electrophysiological studies of cochlear potentials were done by round window membrane electrode and intracochlear electrode recordings in anesthetized guinea pigs. Pathological studies were done by surface preparations of the guinea pig organ of Corti.

MAJOR FINDINGS: Studies done thus far indicate that ethacrynic acid and sodium salicylate potentiate each other's ototoxic effects.

After intravenous administration of sodium salicylate (100 mg/kg body weight) a small drop in the endocochlear potential occurred. Two hours later, intravenous administration of ethacrynic acid (10 mg/kg body weight) produced a large and rapid drop in this potential, not reversible after one hour. Ethacrynic acid by itself causes only a small drop in the potential. By the use of surface preparations of the guinea pig organ of Corti, it has been possible to demonstrate a potentiation by pathological criteria. Two-thirds of guinea pigs treated with daily doses of ethacrynic acid (5 mg/kg body weight) intra-

peritoneally and sodium salicylate (100 mg/kg body weight/day) subcutaneously for 1, 2 and 4 weeks develop more damage to the organ of Corti compared with animals treated with either drug alone. However, as yet, these differences are not statistically significant.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elucidation of any potentiation of ototoxic effects of aspirin and ethacrynic acid has significance in two ways. In the practical sense, such information would be of value to practicing physicians especially internists (rheumatologists, cardiologists), pediatricians and otolaryngologists. In a more theoretical sense, it would provide a better understanding of cochlear pathophysiology.

PROPOSED COURSE: Further pathological studies are to be performed to determine if a significant potentiation exists. Electronmicroscopic studies will also be done to localize the site of the initial lesions. A clinical study using healthy volunteers will be undertaken. This will consist of complete audiological evaluation to be performed by Dr. William G. Thomas of the Division of Otolaryngology, University of North Carolina School of Medicine, on volunteers prior to, during and after receiving both aspirin and ethacrynic acid.

Serial No.: NIEHS-RSB-015
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Incubator Noise and Possible Deafness"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: J. Farmer, M.D.

COOPERATING UNITS: Division of Otolaryngology, Duke University

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives are to answer two questions--1) What noise levels are present in functioning pediatric incubators? and 2) Are such noise levels damaging, or potentially damaging, to the hearing mechanism?

METHODS EMPLOYED: A sound level meter with octave band analyzer was used to measure sound pressure levels in functioning infant incubators. The following sound measurements were made for each incubator and for the background noise: A-weighted sound level, unweighted (linear) sound level, and an octave band frequency analysis with center frequencies of the bands being 31.5, 63, 125, 500, 1000, 2000 and 4000 Hz.

MAJOR FINDINGS: The frequency distribution of the incubator noise followed a consistent pattern among incubators with peak levels between 31.5 Hz and 250 Hz. Above 250 Hz, there was a rapid drop off in sound pressure level so that 99% of the sound energy was distributed below 500 Hz. The average A-weighted and unweighted sound pressure levels are 57.7 dB and 74.5 dB respectively.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: According to present damage-risk criteria for exposure to noise, the above sound levels would not be considered a risk to hearing. However, considering that present damage-risk criteria are based on studies using adult humans and not newborns, that the infant in the incubator is exposed to continuous noise and not intermittent as was used in establishing the present criteria, that con-

tinuous noise is known to be more ototoxic than intermittent, and that infants are often exposed to ototoxic antibiotics, which are known to potentiate the ototoxic effects of noise, such noise levels as were found can be considered as possibly damaging to the inner ear.

PROPOSED COURSE: Noise exposures of newborn and adult guinea pigs will be done; and the inner ears will be examined pathologically to detect whether newborn mammalian cochleas are more, less or equally sensitive to the ototoxic effects of noise.

Serial No.: NIEHS-RSB-016
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of 2450 MHz Microwave Radiation on Biological Material"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. E. Hamrick, Ph.D.

OTHER INVESTIGATORS: D. I. McRee, Ph.D. and P. J. Walsh, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects.

METHODS EMPLOYED: Several strains of bacteria and one bacteriophage have been exposed to 2450 MHz microwaves at power levels sufficient to maintain a temperature of 37°C. Growth rate curves and bacteriophage lysis time have been compared in exposed and control situations. DNA, other cell components and tissue culture cells may be exposed and studied later.

MAJOR FINDINGS: Studies have been made which suggest that "non-thermal" microwave fields (fields which do not significantly increase the temperature of the specimen) at various frequencies interact with intracellular components to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified and, in many cases, the exposure parameters are not known quantitatively. Many of the effects appear to be frequency dependent. For example, the growth rate of Escherichia coli has been shown to be enhanced or retarded depending on frequency in the range of 65-75 GHz. When Escherichia coli and Pseudomonas aeruginosa were exposed to 2450 MHz microwaves at 60 mW/cm^2 and held at 37°C., no effects on growth rate were observed. In addition, no effect was observed on the lysis time of bacteriophage T-2 under similar conditions. Thus far, our studies indicate that if there

are "non-thermal" effects at 2450 MHz, then these effects are relatively small in the systems we have investigated. The test systems used, growth rate and phage production and lysis time, are very sensitive to disturbing influences because the interaction of many cellular components is involved. However, even though these systems did not show effects, it is not possible to say there are no effects other than that due to temperature increases because another biological system may respond differently.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to determine if "non-thermal" effects are present, their magnitude with respect to thermal and if there is any interaction between thermal and "non-thermal" effects. Thermal effects on biological materials have been extensively studied; consequently, it is very important to evaluate the role of "non-thermal" effects. If these are minor, then hazard analysis can be pursued on the basis of known results of subjection of biological specimens to temperature increases.

PROPOSED COURSE: The proposed course is to complete the exposure studies with *Escherichia coli* and the T-2 bacteriophage at 37°C. Further study can be made with these systems at higher temperatures to determine if the thermal effects are influenced by exposure to 2450 MHz microwave radiation. Plans are being made to study the thermal denaturation of exposed and control DNA solutions. Other biological systems which have been implicated as exhibiting "non-thermal" effects are also being considered for exposure.

Serial No.: NIEHS-RSB-017
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Determination of Trace Elements in Biological Samples"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: P. J. Walsh, Ph.D. and P. Hamrick, Ph.D.

OTHER INVESTIGATORS: N. Underwood, Ph.D., University of North Carolina

COOPERATING UNITS: Department of Environmental Sciences and Engineering, University of North Carolina

MAN YEARS

Total:	0.5
Professional:	0.4
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: This project was initiated to develop quantitative techniques for the determination of trace amounts of elements, singly and in combination, in biological samples.

METHODS EMPLOYED: X-ray emission spectrometry is a useful technique for the determination of elements in biological samples. It has the advantage of speed of analysis bacause of its ability to analyze many elements simultaneously and its high detection efficiency. In recent years, the combination of semiconductor detectors with field-effect-transistor input preamplifiers and the optimization of that combination has yielded the resolution necessary to allow separation of the characteristic X-rays for all elements above atomic number 12.

MAJOR FINDINGS: We have developed techniques that promise to extend the sensitivity of X-ray emission spectrometry into the parts per billion range.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aside from its value as a conventional analytical tool, X-ray emission spectrometry offers the possibility of in vivo studies in animals and man ranging from the study of the distribution of iodine in thyroid glands to measurement of deposition of aerosols in the respiratory tract. A variety of studies normally carried out by the use of radioactive tracers appear within the capabilities

of X-ray emission spectrometry.

PROPOSED COURSE: We plan to continue the development of X-ray emission spectrometry as an analytical tool and, in conjunction with NIEHS-RSB-001 and the Lovelace Foundation contract, to explore its application to the determination of aerosol deposition in models and *in vivo*.

Serial No.: NIEHS-RSB-018
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "A Cross-Sectional Area Measuring Device for Whole Biological Samples"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: David A. Dahl and R. H. Wyatt, Ph.D.

OTHER INVESTIGATORS: H. Posner, Ph.D., P. Browning, T. Orr

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The objective is the design and construction of an electro-mechanical instrument for measuring the cross-sectional areas of a whole, unsectioned biological specimen (or any small object of irregular shape) as a function of the specimen length. Moreover, the feasibility and the usefulness of this device are to be investigated.

METHODS EMPLOYED: The operation of the device is based on a fundamental principle of fluid mechanics: any body immersed in a liquid is acted on by an upward buoyant force equal to the weight of the displaced liquid. If an object is immersed slowly into a fluid at a constant velocity, then the acting buoyant force, or the apparent weight loss of the object, is given as follows:

$$W_b(x) = \gamma V(x) = \gamma \int_0^x A(\phi)\, d\phi$$

where: $W_b(x)$ = buoyant force; γ = density of fluid, a constant; $V(x)$ = volume of fluid displaced; x = distance from bottom of object to fluid surface; $A(x)$ = cross-sectional area of object in a plane parallel to fluid surface; and ϕ = variable of integration. Now, differentiating, we have for every x at which $A(x)$ is continuous

$$\frac{dW_b(x)}{dx} = \gamma A(x)$$

Thus, the rate of change of the buoyant force of the object with respect to x is proportional to the area of the object at the fluid surface plane. Because the velocity is constant, x = vt, and thus dx = vdt (v = velocity, t = time). Therefore, a derivative with respect to time is proportional to a derivative with respect to x. Thus,

$$\frac{dW_b(x)}{dt} = \gamma v A(x)$$

Now, continuously weighing the object during immersion, its variable or apparent weight, W_t, is related to the buoyant force and to the object's true weight, W_o, as follows

$$W_t = W_o - W_b$$

Therefore,

$$\frac{dW_t(x)}{dt} = -\frac{dW_b(x)}{dt}$$

which gives a simple expression of proportionality for relating area to weight.

$$A(x) \propto \frac{dW_t(x)}{dt}$$

Electromechanical techniques are employed for sensing and processing the continuous weight signal as the object is slowly lowered into the fluid filled vessel. A mechanical, motor-driven apparatus is used for lowering the object; digital micrologic circuitry allows much of the motor control needed to be performed automatically. To date, water is the only fluid to be used in the immersion vessel. A linear, unbonded, strain gage transducing cell senses the weight signal. The four elements of the cell form a fully active, purely resistive, wheatstone bridge circuit, operating on direct current. The transducer signal is then fed to a modular, differential amplifier employing differential input circuitry with high common mode noise rejection and extremely high input impedance. The output signal voltage is developed single-ended with respect to system common; it is then differentiated by a circuit utilizing operational amplifiers, and compensation for capacitor leakage is also incorporated. The signal from the differentiator is filtered by a special 5.0 Hz low-pass filter; this filter has a linear phase characteristic for negligible signal distortion. The signal from the filter is then put on a wide display, strip-chart recorder where system calibration is performed. The present design of the instrument will yield a device for measuring area cross-sections on objects weighing up to 50 or 60 gms. and having a gross shape-volume characteristic of up to something like a small pear or an apple.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This instrument should enable our local scientists to obtain length versus area characteristics on whole, unsectioned, biological samples of various types

(e.g., fetuses, tumors, etc.), at a large savings in man-hours. A possible application of the instrument is in the study of biological objects with abnormally unsymmetrical morphology, e.g., achondroplasia, micromelia and swellings of various types.

PROPOSED COURSE: The construction of the instrument is nearing completion, at which time tests on performance and usefulness will be conducted. These will include tests for: 1) determining the useful limits of the device, 2) sensitivity, 3) linearity and 4) fluid surface tension and viscous friction effects. Also, if desired, circuitry could be added to incorporate additional characteristic readouts like volume, weight and specific gravity (specific gravity is a potential indicator of lipid or water content in a tumor).

Serial No.: NIEHS-RSB-019
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Investigation of Microwave Radiation Effects on Neurological Functioning"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. H. Wyatt, Ph.D.

OTHER INVESTIGATOR: D. I. McRee, Ph.D.

COOPERATING UNIT: Duke University

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The project objective is an attempt at determining more fundamental reasons and mechanisms underlying the neurological effects of microwaves by studying the effects on basic neural structures.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The hazards of microwave radiation are a priority matter of current concern for NIEHS. The study of biological effects caused by microwave radiation forms the basis for determining safe levels of exposure. Presently, the safe level of continuous exposure in the U.S.A. is 10 mW/cm^2. This is based on one underlying assumption: namely, that the possible damage due to microwave radiation exposure is of a thermal nature. However, many other experiments, especially from Soviet-bloc nations, report indications of nonthermal, neurological effects like headaches, fatigue, hyperactivity, altered brain wave patterns and changes in the functional state of nerves, e.g., induced transmembrane potentials. These observations occurred at radiation levels below the extant U.S.A. safe levels. The question of the nonthermal influence of microwaves is the principle one in the problems and consequences of the biological effects of microwaves, and it must be resolved before the degree of the hazard posed to man is determined.

PROPOSED COURSE: After studying the literature further, it is planned to set up some instrumentation for studying nerve cells subjected to microwave radi-

ation. A variable frequency microwave exposure system is in the process of calibration here. The mechanism by which electromagnetic fields might influence neuroelectric processes has not been established. Aspects to be considered are induced transmembrane potentials, firing pattern activities, conduction rates of action potentials, threshold excitability and electrical models of neurons.

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

A number of changes of a significant nature have occurred in the intramural research program. The Mutagenesis Branch, under Dr. Frederick de Serres, has been established. This Branch is expected to be the national focal point for research in this area. Dr. Robert Dixon has been appointed Chief of the Pathologic-Physiology Branch. This Branch will not only fulfill the implicit service functions but will also investigate problems related to the environmental biology of environmental pollutants.

The size of the intramural program is still inadequate to function properly. It is too small to be able to mount effective, long-term programs aimed at understanding and predicting problems of the effect of environmental agents on human health and at the same time responding appropriately to the urgent problems of today. Nonetheless, progress has been made.

The thrust of the Mutagenesis Branch will be focused on the evaluation and definition of risks to both human germinal and somatic tissues. The Mutagenesis Branch has already addressed itself to the problem by: (1) attempting to identify specific mutagenic hazards in man's environment; (2) developing new and relevant mutational assay systems; (3) developing a better understanding of the molecular basis of mutation; and (4) understanding the induction processes attributable to chemical and physical challenges.

A simple and highly useful assay system has been developed to study the effects of important environmental chemicals on the repair of genetic damage in human cells in tissue culture. The demonstration that N-hydroxy compounds interfere with the essential processes of the assay is of utmost importance in interpreting results derived from the employment of specific chemicals which form a ubiquitous class of carcinogens and proximate carcinogens. The correlations drawn and comparisons made between chemical and radiation damage will serve to make the literature on this subject far more comprehensible.

A comprehensive study of repair events in mouse lymphoma cells is an important ongoing project which lends necessary support to our continuing effort to develop more meaningful mutational test systems employing somatic cells. Only by fully appreciating the precise nature of those events upon which mutation is predicated can a true understanding be provided as to the relevance and significance of mutational assay systems. For this reason, we have undertaken highly detailed studies of the quantitative and qualitative nature of both repair and replication.

A number of methods have now been developed and refined to assess the chemical nature of genetic damage. Furthermore, it is now possible, with certain chemical mutagens, to detect genetic damage to DNA at levels of sensitivity exceeding that normally provided by the use of radioactive isotopes which often are not available for these studies.

The most notable accomplishment has been the development and utilization of a new mutational assay system in mouse lymphoma cells. This work has led to the identification of a drug, used extensively in Africa and South America, as being potently mutagenic to mammalian cells.

The successful development, in mouse lymphoma cells, of two specific loci, one of which is autosomal (TK) and the other X-linked (HGPRT), should contribute much to the identification of mutagenic hazards in the environment as well as to provide an improved understanding of the types of mutations which occur in somatic cells in mammals and the mechanisms by which they accur. Considerable progress has been attained towards an understanding of G6PD variant cells in human peripheral blood. This work is being continued and other loci are also being explored pursuant to the development of cytochemical methods for assessment of somatic mutant frequencies among human populations.

Development has continued on the opossum as a Marsupial model system which will be useful in the investigation of a large variety of physiologic, pharmacologic, pathologic, and toxicologic mechanisms. The Marsupial is at the inframammalian position in the evolutionary scale and is endowed with a number of developmental characteristics which include: (1) a gestation period of 12-3/4 days; (2) no placenta; (3) intrapouch development as an extension of intrauterine development; (4) markedly delayed development of the nervous and respiratory systems; (5) "simplified" thyroid, pituitary, and brain; (6) anomalous iodine transport and storage; (7) extreme tolerance of developing young to high CO_2 levels; and (8) a markedly increased growth rate during the first year of life. All of these characteristics account for the uniqueness of this model system and present many useful areas for the study of environmental hazards. These animals are being utilized experimentally not only by members of the Pathologic Physiology Branch but also, when available, by the Institute in general.

Other research projects in the pathologic-physiology area include investigation of the pathogenesis of heavy metal toxicity and the identification of the toxic cellular effects of heavy metals, particularly the adaptation of detoxifying mechanisms and the other metabolic factors which influence pathologic effects. A study of the toxicity of orally ingested NTA, a chelating agent which is used currently as a phosphate replacement in the manufacture of detergents, has demonstrated a hyperglycemic effect in rats and also characteristic vacuolar lesions occurring in the renal proximal tubules of NTA treated animals.

A large variety of studies in an attempt to assess many of the potential toxicological hazards associated with methylmercury is underway. These investigations include: (1) a comparison of conditioning behavior in rats treated *in utero* with methylmercury and nontreated rats; (2) the effects of methylmercury exposure via the milk in nursing neonate rats; (3) the morphologic lesions in the rat kidneys associated with acute and chronic methylmercury poisoning; (4) the general pathologic effects of methylmercury intoxication in rats;

(5) the comparative pathology of acute methylmercury intoxication in guinea pigs; (6) an evaluation of the potential antidotal effect of glutamate therapy in methylmercury poisoning of adult male rats; (7) the neuropathology of subacute exposure to methylmercury; (8) the metabolism of methylmercury hydroxide by the nervous system; (9) the function and pathology of methylmercury hydroxide relative to the central nervous system; and (10) an assessment of the neurotoxicity with pathologic and physiologic correlation of methylmercury in adult rats. These studies will be of definitive value to the overall understanding of methylmercury intoxication. However, it must be recognized that the parenteral route of administration and the high acute and subacute doses of methylmercury used in these experiments make an extrapolation of these data to the human situation difficult. These investigations are being further refined using oral administration and low doses over a long period of time.

The effects of environmental chemicals on the metabolism of foreign and endogenous substrates and hepatic microsomal drug metabolism is another important area. These include the effects of heavy metals on hepatic microsomal enzymes; the alterations in liver microsomal and lysosomal β-glucuronidase by compounds which induce drug metabolizing enzymes; the metabolism of some carbamate pesticides by liver, kidney, lungs, stomach, intestines, and blood; the effect of chlordane and methylmercury on the metabolism of carbamate pesticides; the purification and determination of enzymic activity of microsomal liver UDP-glucuronyl transferase following treatment with piperonyl butoxide; and the effects of methylmercury on serum and brain cholinesterase activity.

A number of new projects have just been initiated during the past month. These studies include the investigation of mechanisms of drug secretion (renal and biliary) and the effect of environmental agents on such processes; the biochemical mechanisms of spermatogenesis and the toxic effect of exogenous chemicals on male reproduction; the biochemical mechanisms associated with oogenesis and the toxic effect of exogenous chemicals on female reproduction; the effect of prenatal exposure to chemicals on the subsequent reproduction performance of the offspring; and the carcinogenic effect of prenatal exposure to environmental chemicals.

In the mycotoxicology program, a new toxin from Alternaria tenuis, strain M_1, was isolated and its structure determined except for the alternative placement of a hydroxyl and methoxyl group. The compound is an analogue of alternariol monomethyl ether. It is a member of a family of α-pyrones produced by this organism, apparently through the mechanism of polyketide condensation.

Two new moderately toxic compounds were isolated from another Alternaria sp.; e.g., A. mali, strain A_3. Both of these have been partially characterized. They have the empirical formulae $C_{20}\ H_{16}$ and $C_{20}\ H_{14}\ O_6$. The formulae and various physical-chemical characteristics suggest that they are closely related compounds differing only with respect to two protons. Their characteristics closely parallel those of xanthones and anthones. Determination of precise structures, however, requires further work and probably X-ray analysis in order to establish the sites of hydroxyl groups, double bonds, and the fourth ring. The compounds do not correspond precisely in physical characteristics to any xanthones or anthones available as models.

The screening program for new toxigenic fungi yielded a number of toxic isolates from commercial macaroni, rice, corn meal, and wheat flour. The toxins from these will be studied as time permits. It may be significant that one of the isolates from macaroni appears to produce aflatoxins B_1 and B_2 and has been tentatively identified as Aspergillus flavus.

Studies were continued to determine the influence of some commercially important fungicidal compounds on the morphological characteristics and toxin producing capabilities of Alternaria mali, strain A_3. Six morphological variants were found upon treatment with zineb, five with maneb, four with nabam, three with ferbam, two with thiram, and two with captan. Because the variants were derived from a single nuclear type and no variants were observed in the controls, they are considered to be mutants. Toxin production by the variants differed widely, from one isolate which was as toxic to mice as the wild type (LD_{50} 300 mg/kg) to one which was not lethal at 2400 mg/kg. It was found also that vapors from deposits (presumably decomposition products) of all the fungicides greatly reduced toxin production by the fungus.

Recent reports have indicated that inhibition of protein synthesis may be a primary factor in the effects of mercurials on the central nervous system. Work reported last year showed that sensitivity of oxidative phosphorylation to mercurials is as great or greater than that of protein synthesis and, therefore, may be involved in or account completely for observed effects on protein synthesis in the central nervous system. The experiments this year show that effects of different mercurials on protein synthesis in different organs is a simple function of mercury levels, which vary from organ to organ because of differential distribution. Following administration of methylmercury at dosages resulting in liver or brain levels below 0.4 µg/mg protein, protein synthetic activity was increased; in the kidney where mercury accumulated, protein synthesis was inhibited. In cell free systems, where distribution and stability differences are largely negated, no appreciable difference in the concentration of mercurials necessary to inhibit protein synthesis was found.

Research in the animal science and technology area involving the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) dealt with further characterization of its teratogenicity and toxicity. Using the C57B1/6 mouse, the specific period of fetal development which is susceptible to TCDD was investigated. Extension of these studies revealed that neonates develop polycystic kidneys. This is only the second example of chemical exposure to a foetus resulting in tubular dysgenesis. Adult oral toxicity experiments indicate early and progressive effects on liver function.

Several studies dealing with the effects of 3 purified polychlorinated biphenyls (PCBs) are in progress. The pathological, immunological, and hematological and clinical chemical parameters are being investigated. There appears to be a positive correlation between the severity of the toxicity and increased chlorine content of the biphenyl. The interrelationship of an environmental contaminant and an infectious disease is being investigated utilizing various dose levels of PCBs on mice inoculated with Moloney leukemia virus. Studies on the effect mobilization of fat stores may have on animals which have received chronic doses of PCBs is also planned.

A disease due to the protozoan parasite Encephalitozoan cuniculi continues to interfere with interpretation of brain and kidney lesions seen in the rabbit species. In a cooperative effort with scientists at Ohio State University, the Animal Science and Technology Branch has commenced a program designed to characterize the transmission and pathogenesis of the disease. These experiments, coupled with refinement of a skin test recently developed by OSU, will result in procedures being developed leading to eradication of the disease.

A study of the effect of household detergents on the rabbit eye was prompted by changes in product formulations. It was dramatically shown that some phosphate substitutes have severe corrosive properties and accidental introduction to the eye could produce impaired vision.

Hycanthone methane sulfonate, a drug used in schistosomiasis therapy, was examined for teratogenic potential following several reports citing it as a mutagen. It is also a potent teratogen; once again depicting a correlation between a mutagen and a teratogen.

Evaluation of the captive-born opossum as a research model continued. These animals continue to remain parasite-free in our system of housing. Improved management practices produced a marked decrease in post-weaning mortality and deaths due to endocarditis. Preweaning mortality remains high. Second generation animals had a conception percentage approaching 70 percent.

The polychlorinated biphenyls (PCBs) have been extensively used during the last 40 years, primarily as coolant insulation fluids in transformers and capacitors, heat exchanges, plasticizers, in carbonless paper, printing inks, waxes, paints, and waterproofing compounds. Only recently have aspects of the ecological pervasiveness and concentration in the food chain of the PCBs been recognized. Essentially the same type of residue pattern is becoming apparent for the PCBs as has been found for the persistent organochlorine insecticides. However, compared to the chlorinated hydrocarbons, the definitive mammalian acute, subacute, and chronic toxicity of the PCBs remains poorly known. In order to study these problems, the development of requisite analytical methodology and the synthesis of a number of key purified labeled and unlabeled isomeric chlorinated biphenyls (210 isomers of the PCBs are possible, but only 10-15 isomers are widely used) are necessary. The rates of absorption and elimination of a select number of PCBs, their tissue localization, and identification of their metabolites following oral, i.p., and i.v. administration are being studied by the Analytical and Synthetic Chemistry Branch.

Brain Na-ATPase and Na-independent ATPase represent enzymes associated with the neuronal membrane. Initial studies indicated that reversal of $(Na^{+}+K^{+})$ATPase inhibition by PCBs and DDT could be effected by crude commercial phospholipid preparations. The responsible component was identified as phosphatidyl serine. Phosphatidyl ethanolamine and phosphatidyl inositol inhibited the enzyme and offered no protection. The dose-response relationships to the chlorinated hydrocarbon pesticide inhibitors were found to be quantitatively similar to those involving oleic acid (tris salt); and because inhibition by both pesticide and fatty acid may be prevented or reversed by phosphatidyl serine, it is tentatively suggested that these two classes of inhibitors probably effect inhibition by emulsifying or otherwise interacting with phospholipids which are required for normal enzyme activation.

The aromatic dicarboxylic esters such as the phthalic acid esters, e.g., di(2-ethylhexyl) [DHP] and dioctyl [DOP] phthalates, are among the most important industrial chemicals employed. Although they have enjoyed extensive utility for three decades, primarily as plasticizers for a variety of films and plastics, it has been only within the last several years that their migration from plastics into human tissue as well as their increasing occurrence in the ecology have been reported. Hence, there is increasing concern regarding the consequences in man of chronic ingestion, absorption, and/or inhalation of low levels of a variety of phthalate esters. The development of requisite analytical methodology, the preparation of purified labeled ^{14}C-DHP and DOP, and the subsequent elaboration of their transport and mammalian metabolism are underway.

Mirex is an insecticide used for the irradication of the fire ant in the Southeastern United States. However, residues of Mirex have already been found in both shellfish and catfish in fresh water and ocean sources. If Mirex is not biodegradable, its further use could create a major pesticide residue problem. Recent results show Mirex to be remarkably stable. To date, no metabolite of Mirex has been detected in feces, tissues, or urine following oral administration of Mirex-^{14}C in the rat. Mirex was not metabolized by any rat or mouse liver preparation. Pea and bean root preparations known to be biochemically active also failed to metabolize Mirex. Pea and bean plants were found to absorb (and concentrate) Mirex from water at 1, 5, and 10 ppm levels.

The banning of the food additive cyclamate due to its role in bladder tumor induction and the more recent finding of the induction of bladder tumors in the rat following implantation of saccharin pellets have focused on the need to study these compounds and their derivatives. Accordingly, the potential metabolites of saccharin and cyclamate (e.g., N-hydroxy, N-acetoxy, methoxy, nitroso and nitrile, dihydrosacchrin, etc.) are being prepared.

Both in vitro and in vivo studies to date have failed to reveal a metabolite of sacchrin. When fed a dose equivalent to a normal human exposure (1 mg/kg/day) for seven days, both male and female rats rapidly excrete sacchrin, primarily in the urine, without any apparent biological alteration of the molecule. An insignificant amount of this compound is stored in any of the eleven tissues and organs investigated.

An important class of environmental agents is represented by the methylenedioxy moiety which is found widely distributed in essential oils, alkaloids, and other physiologically active compounds of natural origin. Compounds containing this moiety are widely used in insecticides (primarily as synergists), food additives, perfumes, and medicinals. Nitrogen-containing urinary metabolites [e.g., 3-piperidyl-1-, 3-pyrrolidinyl-1-, and 3-N,N-dimethylamino-1-(3',4'-methylenedioxyphenyl)-1-propanones] have been identified following administration of safrole and myristicin to rats; while elemicin and eugenol yield 3-piperidyl-1- and 3-pyrrolidinyl-1-(trimethoxy phenyl) and 1-(methoxy phenyl)-1-propanone derivatives, respectively. The metabolism of these tertiary aminopropiophenones by monoamine oxidases as well as the elaboration of the biosynthesis of piperidine and pyrrolidine is in progress. The synergistic action of methylenedioxphenyl derivatives with various pesticides is attributed to inhibition of insect enzyme systems or prevention of enzyme induction. Pesticide synergists are used in aerosols which are widely employed for home use. Studies are in progress on

the effects of inhaled pesticidal synergists and carbon monoxide on these enzymes. If the mixed-function oxidase enzymes of the lungs serve to protect the lungs and the individual from the effects of inhaled xenobiotics, inhibition of these enzymes by the contents of household aerosols and urban air may play a significant role in lowering the resistance of the exposed individual to the actions of any inhaled xenobiotics.

Interesting results have been obtained by the Pharmacology-Toxicology Branch while comparing detoxication-toxication mechanisms in lung and liver of rabbits and rats and studying how these systems develop with age and respond to exposures of animals to chemicals, pollutants, and/or drugs. The liver has long been thought to be the key organ in the body's metabolism of xenobiotics and steroids. It is becoming obvious that other organs contain systems capable of metabolizing and thereby altering the reactivity of chemicals (and drugs) delivered to them. The Branch's programs are now concerned primarily with determining what kinds of chemical metabolizing systems may be involved in removing or making more toxic those chemicals that are ingested by oral or inhalation routes. Chemicals, drugs, and pesticides often affect liver toxication-detoxication systems and thereby change their own and other chemical's actions and toxicities for the liver and the whole animal. The same is probably true in the lung, but the work so far suggests that there will be marked differences in kinds of chemicals metabolized in lung vs. liver as well as marked differences in effects of chemicals on these metabolizing systems in lung vs. liver. Therefore, drug-chemical-pesticide-pollutant interactions in the lung will produce quite different changes in action and toxicity of a chemical in the lung as compared with the liver.

A lung perfusion apparatus has been developed in which the uptake and release of chemicals delivered to the isolated, perfused lung by blood or by inhalation can be studied. Such experiments can define the parameters influencing uptake, release, and interaction of chemicals in the lung because there are no indirect effects of the rest of the animal's body on this system. A large number of chemicals are concentrated in the lung; however, these initial studies are limited to lung uptake of basic chemicals such as amines. A number of pollutants and pesticides are in this class, as are many important drugs. The localization of basic chemicals in the lung is important to the duration of action and toxicity of many of these chemicals. Yet little is known about this process or the interactions of chemicals at this process.

Another research effort is concerned with the biosynthesis and release of lung surfactant and the changes in these processes with age and with exposure to pollutants. Lung surfactant seems to play a key role in lung pathology in the newborn and in the processes leading to emphysema; studies of the key steps in the synthesis of this material may help in altering that process or preventing its destruction by toxic materials. Another program concerns the role of lung lipids in the storage of fat soluble pesticides and chemicals such as DDT and PCB. This program also interdigitates with a similar effort concerned with pesticide and pollutant uptake by liver lipids and especially the liver lipid body. Storage of fat soluble pesticides and pollutants in the lung and liver probably regulates not only the duration of their stay in these organs, but also their toxic effects in these and other tissues. Kinetics of uptake and release of these materials in lung and liver lipids are being studied in hopes of understanding how these processes may be altered.

Work on inhalation exposures and toxicology has been delayed by late delivery and installation of exposure chambers. Studies performed so far include: (1) the effects of repeated exposure to SO_2 on the ability of lung and liver to detoxify SO_2 by reduction to SO_4^{-2}; (2) effects of single and repeated exposures to benzene or xylene in rats also treated with drugs and chemicals that change benzene or xylene detoxication in liver and lung; and (3) effects of single and repeated exposures of rats and rabbits to selected components of aerosol deodorants. The most interesting studies show that interactions of chemicals and drugs can markedly alter the toxicity of materials inhaled by animals; thus, xylene toxicity is markedly shifted by previous exposure of the animal to chemicals and drugs that affect liver and lung metabolism by xylene. Inhalation of hexachlorophene or aluminum chlorhydrin (components of aerosol deodorants) can affect the lung toxication-detoxication systems at exposures that have little or no histologically observable effects on the lung; i.e., the lung detoxication systems may serve as early warning systems for damage as well as explaining some interactions that are puzzling to classical toxicology.

Work in reproduction and ontogenesis so far has been directed toward setting up the techniques for collecting and storing ova, sperm, and fertilized ova from several species. It is now possible to culture fertilized ova in vitro for several days and reimplant such embryos (having grown in culture to this stage) in foster mothers (pseudo-pregnant) and then subject the newborn animals to examination for teratology, etc. These techniques will allow the study of the effects of precisely defined environmental alterations on reproductive processes up to and slightly beyond the time of placentation. Precisely known amounts of known chemicals or pollutants can be added to the culture or injected into the egg or embryo at known times. Techniques are also being developed to transfer nuclei from such treated cells into normal cell cytoplasms so as to decide where a chemical has had its effect (nucleus or cytoplasm). Such techniques have not been applied to understanding teratology of chemicals or environmental pollutants anywhere. This elegant type study will be coupled with routine, classical investigations of chemicals and mixtures for teratologic effects in animals.

Investigations of statistical procedures for analyzing teratological data were continued by the Biometry Branch. A probabilistic model was developed to examine the possible relationship between the estimated proportions of abnormal and dead fetuses within a given litter. Computer simulations were used to evaluate the robustness of the t-test applied to data in which a large proportion of the animals was unaffected by either of the treatments being compared. Finally, the extent to which the proportion of dead, abnormal, and normal fetuses depends on the number of implantations was determined for three different strains of (control) mice.

The development of new nonparametric procedures for application to survival data is being continued. A probabilistic model has been studied for describing the occurrences of particular diseases in a competing risk framework. Nonparametric techniques are used to estimate the model giving both the disease incidence and cumulative mortality adjusted for competing causes of death. A distribution-free method of estimating non-lethal disease incidences has also been investigated. Finally, methods for testing treatment interactions on general survival times have been developed.

A variable frequency microwave exposure system is being installed and will complement the existing 2450 MHz microwave system developed in the Biomedical Instrumentation of the Research Services Branch. The frequency of the new system can be varied from 100 to 12,000 MHz, and the power level varied from 0.01 to 10 mW/cm^2. Because the interaction of microwaves with matter is frequency specific, the addition of this new system will give greater flexibility in exploring the biological effects of microwave exposure. The development of new and improved dosimetry techniques for microwaves is continuing. Techniques under investigation include the use of thermocouples, thermisters, magnetic probes, and pyroelectric crystals.

ANALYTICAL AND SYNTHETIC CHEMISTRY BRANCH

ANALYTICAL AND SYNTHETIC CHEMISTRY BRANCH
Summary Statement

The programs of the Chemistry Branch are designed to conduct and augment research related to the possibly harmful effects in a wide area of environmental exposures (e.g., pesticides, food additives, trace metals, fungal toxins, and air and water pollutants—including their metabolic and degradation products). They include the chemical analysis of multiple components in the elaboration of synergistic and potentiating effects of these agents.

The Branch is engaged in the development, improvement and standardization of new and improved analytical techniques for the determination of a spectrum of specific metabolic and degradation products arising from inter- and intra-Branch investigations as well as studies of the Branch involving other governmental laboratories. It utilizes diverse analytical procedures consisting of spectroscopy (visible, ultra-violet, infra-red, emission fluorescence, mass and nuclear magnetic resonance) and chromatography (paper, thin-layer, column, radioautography and gas-liquid), using in the latter instance a variety of detectors such as flame, thermal conductivity and electron-capture for greatest efficiency.

Synthetic procedures are employed for preparation of required intermediates and for confirmation of the identity of metabolites and degradation products, as well as for other Branch investigations; e.g., pharmacological, teratological, mutagenic and DNA-binding studies.

The Chemistry Branch is currently engaged in a number of intra- and inter-Branch investigations involving classes of agents recently found to be of pronounced environmental significance. Illustrative of these chemical agents are the polychlorinated biphenyls (PCB's) which are complex isomeric mixtures that are chemically resistant, thermally stable, lipid-soluble and relatively non-biodegradable. Despite their extensive utility during the last 40 years, primarily as coolant insulation fluids in transformers and capacitors, heat exchangers, plasticizers, in carbonless paper, printing inks, waxes, paints and water-proofing compounds, aspects of their ecological pervasiveness and concentration in the food chain have only recently been recognized. Essentially the same type of residue pattern is becoming apparent for the PCB's that has been found for the persistent organochlorine insecticides. However, compared to the chlorinated hydrocarbons, e.g., DDT and congeners whose structural and physico-chemical properties they closest resemble, the definitive mammalian acute, subacute and chronic toxicity of PCB's still remains poorly known. In order to more definitively elaborate these aspects, the Branch is initially engaged in the development of requisite analytical methodology (primarily GLC, mass and NMR spectroscopy) and in the synthesis of a number of key purified labeled and unlabeled isomeric chlorinated biphenyls (210 isomers of the PCB's

are theoretically possible, but approximately 10-15 isomers are found in the most widely used commercial samples, e.g., Aroclors 1221, 1232, 1242, 1248 and 1254). Other studies in progress relate to the elaboration of the in vitro and in vivo metabolism of the PCB's. The latter focus relates to the rates of absorption and elimination of a select number of PCB's, their tissue localization and identification of their metabolites following oral, i.p. and i.v. administration. The interaction of the PCB's with calf brain membranal Na^++K^+-ATPase is being elaborated and compared with the analogous enzymic interactions with the chlorinated hydrocarbons.

Brain Na-ATPase and Na-independent ATPase represent enzymes associated with the neuronal membrane. The Na-ATPase is generally assumed to play a major role in neuronal depolarization-repolarization phenomena in addition to, or in conjunction with, its role in regulating alkali metal ion transport and cellular electrolyte balance. Initial studies indicated that reversal of Na^++K^+-ATPase inhibition produced by DDT and other chlorinated hydrocarbon pesticides could be effected by crude commercial phospholipid preparation; and the component responsible was identified as phosphatidyl serine. Commercial, chromatographically homogeneous preparations of phosphatidyl ethanolamine and phosphatidyl inositol inhibited the enzyme and offered no protection. The dose-response relationships to the chlorinated hydrocarbon pesticide inhibitors were found to be quantitatively similar to those involving oleic acid (tris salt) and because inhibition by both pesticide and fatty acid may be prevented or reversed by phosphatidyl serine, it is tentatively suggested that these two classes of inhibitors probably effect inhibition by emulsifying or otherwise interacting with phospholipids which are required for normal enzyme activation.

A number of chlorinated biphenyls have been studied and found to exhibit properties of interaction with the above ATP-ases which appear to be intermediate between those of DDT and those of oleate or dodecyl sulfate.

The aromatic dicarboxylic esters such as the phthalic acid esters, e.g., di(2-ethylhexyl) (DHP) and dioctyl (DOP) phthalates, are among the most important industrial chemicals employed. Although they have enjoyed extensive utility for three decades, primarily as plasticizers for a variety of films and plastics, it has only been within the last several years that their migration from plastics into human tissue as well as their increasing occurrence in the ecology have been reported. Hence, there is increasing concern regarding the consequences in man of chronic ingestion, absorption and/or inhalation of low levels of a variety of phthalate esters. The Chemistry Branch is engaged in the development of requisite analytical methodology, the preparation of purified labeled ^{14}C-DHP and DOP and the subsequent elaboration of their transport and mammalian metabolism.

Mirex is an insecticide used for the irradication of the fire ant in the Southeastern United States. However, residues of Mirex have already been found in both shellfish and catfish in fresh water and ocean sources. If Mirex is not biodegradable, its further use could create a major pesticide residue problem. Studies are in progress to determine if Mirex undergoes biodegradation and if so, to elaborate the rate and nature of the products via both in vitro and in vivo investigations. Our studies indicate Mirex to be remarkably stable. To date, no metabolite of Mirex has been detected in feces,

tissues or urine following oral administration of Mirex-^{14}C in the rat. Mirex was not metabolized by any rat or mouse liver preparation when incubated with various co-factors for prolonged periods. Pea and bean root preparations known to be biochemically active also failed to metabolize Mirex. Pea and bean plants were found to absorb (and concentrate) Mirex from water at 1, 5 and 10 ppm levels which are likely to be analogous to the situation found in the areas of continued Mirex application.

A number of cyclic and linear metallodithiocarbamates have wide utility as fungicides and rubber accelerators and hence are of environmental significance. Their rates of decomposition as well as the nature of their decomposition products are being elaborated by variable temperature nuclear magnetic resonance spectroscopy.

The increasingly ubiquitous nature of mercury in the environment as well as its conversion to methyl mercury and the latter's apparent toxicological significance is a matter of increasing concern. Studies are in progress to elaborate the mammalian effects of repeated storage of sub-lethal amounts of mercury in terms of tissue storage, identification of the form of mercury stored, rate of storage and/or conversion and concomitant effects on microsomal activity. Analogous studies are in progress relating to the effects of sub-lethal administration of cadmium derivatives.

The banning of the food additive cyclamate due to its role in bladder tumor induction, the metabolic, mutagenic and teratogenic aspects of its metabolites and the more recent finding of the induction of bladder tumors in the rat following implantation of saccharin pellets have focused on the need to study the above compounds and related derivatives for such potential hazards. Accordingly, the preparation of potential metabolites of saccharin and cyclamate, e.g., N-hydroxy, N-acetoxy, methoxy, nitroso and nitrile, dihydrosaccharin, etc., has been in progress because they are vital for the metabolic and toxicologic and DNA-binding studies of the above food additives and their degradation products.

Both *in vitro* and *in vivo* studies to date have failed to reveal a metabolite of saccharin. When fed a dose equivalent to a normal human exposure (1 mg/kg/day) for seven days, both male and female rats rapidly excrete saccharin primarily in the urine, without any apparent biological alteration of the molecule. The rapid excretion of saccharin accounts for the fact that an insignificant amount of this compound is stored in any of the eleven tissues and organs investigated.

An important class of environmental agents is represented by the methylenedioxy moiety which is found widely distributed in essential oils, alkaloids and other physiologically active compounds of natural origin. Compounds containing this moiety are widely used in insecticides (primarily as synergists), food additives, perfumes and medicinals.

Studies are in progress to evaluate whether a potential hazard exists on exposure to these agents and to understand the mechanisms controlling enzymic detoxification of this important class of environmental agents. These studies include the unequivocal identification (by GLC-MS and N.M.R. and I.R.) of the

nature of the metabolites of methylenedioxyphenyl food additives and related compounds (e.g., myristicin, safrole, isosafrole, elemicin, eugenol, cis and trans-asarones), their tissue distribution and routes of elimination following several routes of administration in several species and their effect on microsomal drug-metabolizing enzymes. Nitrogen-containing urinary metabolites, e.g., 3-piperidyl-1-, 3-pyrrolidinyl-1- and 3-N,N-dimethylamino-1-(3',4'-methylenedioxyphenyl)-1-propanones, have been identified following administration of safrole and myristicin, while elemicin and eugenol yield 3-piperidyl-1- and 3-pyrrolidinyl-1-(trimethoxyphenyl) and 1-(methoxyphenyl)-1-propanone derivatives, respectively. The metabolism of these tertiary aminopropiophenones by monoamine oxidases as well as the elaboration of the biosynthesis of piperidine and pyrrolidine is in progress.

The synergistic action of methylenedioxyphenyl derivatives with various pesticides is attributed to inhibition of enzyme systems or prevention of enzyme induction, both concerned with the metabolism of the pesticides. Pesticide synergists are used in aerosols of insecticides which are widely employed for home use. Studies are in progress to elaborate the mixed-function oxidase enzymes of mammalian lung and the effects of inhaled xenobiotics, such as pesticidal synergists and carbon monoxide, on these enzymes. If the mixed-function oxidase enzymes of the lungs serve to protect the lungs and consequently the individual from the effects of inhaled xenobiotics, inhibition of these enzymes by the contents of household aerosols and urban air may play a significant role in lowering the resistance of the exposed individual to the actions of any inhaled xenobiotics.

Dieldrin is representative of a class of persistent pesticides of major importance, the cyclodienes. A study of the metabolism, storage and rates of excretion of dieldrin in the male and female rat following administration of doses possibly analogous to that resulting from environmental exposures is in progress. In addition to aldrin-trans diol, the cis isomer (previously unreported) has been isolated as a metabolite of dieldrin, its enzymic conversion to the trans diol demonstrated and the "epimerization" confirmed by mass spectral, GLC and TLC analysis.

Another aspect of the activities of the Chemistry Branch relates to the elaboration of potential environmental hazards from natural products. Studies to determine whether exogenous prostaglandins should be considered environmental factors, either relative to ingestion or topical exposure, as well as to determine whether exogenous prostaglandins play a significant role in the response of animals to drugs, pesticides, antigens, etc., are in progress. Techniques for the recovery and identification of sub-microgram quantities of newly-synthesized prostaglandins as well as for the isolation of clean, metabolically active mast cells have been developed or refined. Prostaglandins of the E Type have been found to stabilize mast cells against spontaneous release of histamine, while A, B and F prostaglandins have no discernible effect. Studies are in progress to further elaborate the role of prostaglandins in anaphylactic response.

The development of new and the improvement of established techniques is an integral requirement for the solution of diverse problems confronting scientists at the Institute. The development of analytical methodology includes: the

characterization of the impurities of pesticidal synergists such as piperonyl butoxide; the qualitative and quantitative analysis of the isomeric chlorinated biphenyls; the characterization of synthesis products of phthalate esters and their potential metabolites; the determination of total, organic and inorganic heavy metals (e.g., mercury, cadmium) in biological tissues; the analysis of the mutagenic anti-schistosomal agent hycanthone in mouse lymphoma cells and mammalian tissue; and the analysis of Mirex in animal and plant tissue.

A number of contracts are also currently under the sponsorship of the Chemistry Branch and these include: "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants" (Battelle Pacific Northwest Laboratories), "Long-Range Toxicologic Effect of Metal-Containing Dithiocarbamate Fungicides" (Midwest Research Laboratories), "The Interaction of Specific Air Pollutants with Lung Lipids" (Southwest Research Instutute), and "Influence of Microorganisms and Other Soil Factors on Degradation of Certain Insecticides" (U. S. Forestry Service).

Dr. Fishbein is authoring a four-volume series, "Chromatography of Environmental Hazards", for Elsevier Press and has completed the first two volumes (Vol. I. Carcinogens, Teratogens and Mutagens, and Vol. II. Metals, Gaseous and Industrial Pollutants). He has participated in an Interagency PCB Task Force that was formed to evaluate the potential hazards of the PCB's and has summarized their findings in a "PCB's and the Environment" report. He has also participated in presenting invited papers at the Fifth International Congress of Rural Medicine, Varna, Bulgaria, May 10-15, 1972. Dr. Fishbein is the NIEHS Project Officer for the contracts, "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants" and "Long-Range Toxicologic Effect of Metal-Containing Dithiocarbamate Fungicides".

PUBLICATIONS *

Fishbein, L.: Chromatography of Environmental Hazards, Vol. I, Carcinogens, Mutagens and Teratogens, Elsevier, Amsterdam, 1972, pp. 499.

Fishbein, L.: Chromatographic and biological aspects of inorganic mercury. Chromatog. Revs. 15: 195-238, 1971.

Fishbein, L.: Pesticidal, Industrial, Food Additive and Drug Mutagens. Fogarty International Center Proceedings, No. 9. "Mutagenic Effects on Environmental Contaminants", Washington, D. C., March 29-31 (1971), Academic Press, New York, 1972.

Fishbein, L.: in "Chemicals and Future of Man", Hearings of the Sub-Committee on Executive Reorganization and Government Research, Washington, D. C., April 6, 7 (1971).

Fishbein, L.: "PCBs and the Environment", Interagency Task Force Report, National Technical Information Service, Springfield, Va., April, 1972.

*Excludes those publications of the Branch Chief that are listed with individual project reports.

Fishbein, L.: Chromatography of Environmental Hazards, Vol. II, Metals, Gaseous and Industrial Pollutants, Elsevier, Amsterdam. (In Press).

Fishbein, L.: Chromatographic and biological aspects of polychlorinated biphenyls. Chromatog. Revs. (In Press).

Fishbein, L. and Albro, P. W.: Chromatographic and biological aspects of phthalate esters. Chromatog. Revs. (In Press).

Fishbein, L.: Chromatographic and biological aspects of radiopharmaceuticals. I. Iodinated derivatives. Toxicol. Env. Chem. Revs. (In Press).

Fishbein, L.: Natural non-nutrient substances in the food chain. Sci. Total Environ. (In Press).

Fishbein, L. and Flamm, W. G.: Potential Environmental Chemical Hazards. I. Drugs. Sci. Total Environ. (In Press).

Fishbein, L. and Flamm, W. G.: Potential Environmental Chemical Hazards. II. Feed medicants and pesticides. Sci. Total Environ. (In Press).

Fishbein, L. and Flamm, W. G.: Potential Environmental Chemical Hazards. III. Industrial and miscellaneous agents. Sci. Total Environ. (In Press).

Fishbein, L., Falk, H. L., Fawkes, J. and Jordan, S.: The Metabolism of ^{14}C-Piperonyl Butoxide in the Rat. IUPAC - 2nd International Congress of Pesticide Chemistry, Tel-Aviv, Israel, Feb. 21-26 (1971). In Press).

Wilson, W. E., Fishbein, L. and Clements, S. T.: DDT: Participation in ultraviolet detectable charge-transfer complexation. Science 171: 180-182, 1971.

Albro, P. W. and Fishbein, L.: Isolation and characterization of 5, 8, 12-trihydroxy-trans-9-octadecenoic acid from wheat bran. Phytochem. 10: 631-636, 1971.

McKinney, J. D., Fishbein, L. and Barling, L.: Selective complexation of impurities in the electron capture GLC determination of some chlorinated polycyclodiene metabolites and derivatives. Bull. Env. Contam. Toxicol. 7: 2-8, 1972.

Albro, P. W. and Fishbein, L.: Quantitative and qualitative analysis of polychlorinated biphenyls by GLC and flame ionization. I. One to three chlorine atoms. J. Chromatog. (In Press).

McKinney, J. D., Oswald, E. O., Fishbein, L. and Walker, M.: Mechanisms of formation of Mannich bases. Bull. Env. Contam. Toxicol. (In Press.)

Bick, M. and Fishbein, L.: Effect of certain drugs and environmental agents on microsomal enzyme systems. Sci. Total Environ. (In Press.)

Bick, M. and Fishbein, L.: Inductive effects of piperonyl butoxide on microsomal activity. Sci. Total Environ. (In Press.)

Serial No.: NIEHS-ASC-003
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Myristicin and Congeners"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: E. Oswald, Ph.D.

OTHER INVESTIGATORS: B. Corbett, M. P. Walker, L. Fishbein, Ph.D.

COOPERATING UNITS: Pathologic Physiology Branch

MAN YEARS

Total:	2.0
Professional:	0.5
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: Substituted allyl and propenyl benzene derivatives are widely distributed in nature, e.g., in nutmeg, parsley, parsnip, carrots and bananas, as well as in processed tobacco and flavoring agents. In order to determine whether these environmental agents constitute a potential hazard, investigations are in progress to isolate and identify the various urinary metabolites of myristicin, safrole, asarone, beta asarone and dihydrosafrole following administration by various routes to the rat.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), NMR, I.R. and mass spectroscopy.

MAJOR FINDINGS: Following oral or intraperitoneal administration of myristicin, safrole, isosafrole, elemicin, eugenol, asarone (trans) or beta asarone (cis) to male rats, basic ninhydrin positive materials are excreted in the urine. There is an apparent requirement of a side chain double bond for the production of these materials with greater enhancement by the trans isomer than the cis.

Partial characterization of two ninhydrin positive metabolites of safrole indicate the following: (1) the methylenedioxy ring is intact in both, (2) the aromatic protons of both materials are similar to those of safrole, and (3) both of these polar basic urinary metabolites degrade to a very non-polar carbonyl compound which is ninhydrin negative and yields hydrazones with 2,4-dinitrophenylhydrazine.

Although the above urinary metabolites of safrole bear resemblance in their basicity and ninhydrin positive character to those obtained following administration of 3,4-methylenedioxy amphetamine, the safrole metabolites are more reactive and labile than the amphetamines.

Complete structural identification indicates that three nitrogen-containing metabolites of safrole are excreted in the urine of rats and/or guinea pigs following oral or i.p. administration. The major safrole basic ninhydrin positive metabolites of the guinea pig and rat are 3-N,N-dimethylamino-1-(3',4'-methylenedioxyphenyl)-1-propanone and 3-piperidyl-1-(3',4'-methylenedioxyphenyl)-1-propanone, respectively. In addition, the rat also excretes the above N,N-dimethylamino ketone and trace amounts of 3-pyrrolidinyl-1-(3',4'-methylenedioxyphenyl)-1-propanone. All three tertiary aminopropiophenones decompose to form 1-(3',4'-methylenedioxyphenyl)-3-propen-1-one.

The major basic ninhydrin positive urinary metabolite of myristicin in the rat is 3-piperidyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone. In addition, the rat and guinea pig excrete trace quantities of the pyrrolidinyl ketone and piperidyl ketone, respectively. Similarly, the major basic ninhydrin positive urinary metabolite of elemicin in the rat is 3-piperidyl-1-(3',4',5'-trimethoxyphenyl)-1-propanone with smaller amounts of the 3-pyrrolidyl-1-(3',4',5'-trimethoxyphenyl)-1-propanone. Very similar nitrogen-containing metabolites of eugenol are also excreted by the rat. In contrast to the urinary basic metabolites of safrole, no detectable quantity of the N,N-dimethylamino ketone was present in either rat or guinea pig urine after administration of myristicin; the guinea pig upon administration of safrole excreted only the substituted 3-N,N-dimethylamino propiophenones.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Unequivocal identification of the metabolites and knowledge of the rates of elimination of these environmental agents are essential in determining whether the exposure or ingestion of these agents and related compounds constitutes a potential hazard.

PROPOSED COURSE: (a) Complete identification and characterization of both the basic ninhydrin positive and non-basic urinary metabolites of safrole, myristicin, elemicin, eugenol and the asarones, (b) preparation of C^{14}-labeled myristicin, safrole, elemicin and eugenol, and (c) elaboration of these drugs following introduction into whole animals and finally cell-free systems.

PUBLICATIONS

Oswald, E. O., Fishbein, L. and Corbett, J.: Metabolism of naturally-occurring propenyl benzene derivatives. J. Chromatog. 45: 437-445, 1969.

Oswald, E. O., Fishbein, L., Corbett, B. J. and Walker, M. P.: Identification of tertiary aminomethylenedioxy propiophenones as urinary metabolites of safrole in the rat and guinea pig. Biochem. et Biophys. Acta 230: 237-247, 1971.

Oswald, E. O., Fishbein, L., Corbett, B. J. and Walker, M. P.: Urinary excretion of tertiary amino methoxy methylenedioxy propiophenones as metabolites of myristicin in the rat and guinea pig. Biochim. et Biophys. Acta 244: 322-328, 1971.

Oswald, E. O., Fishbein, L. and Corbett, B. J.: Metabolism of naturally occurring propenyl benzene derivatives. II. Separation of tertiary amino propiophenones by gas-liquid chromatography. J. Chromatog. (In Preparation).

Serial No.: NIEHS-ASC-006
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Development of Analytical Methodology"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; E. O. Oswald, Ph.D.; R. Thomas; J. Fawkes; A. Latimer

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	0.8
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVES: To develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which no analytical methodology, insufficiently specific or sensitive methodology, or no methodology we are equipped to utilize are presently available; (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR,UV,Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS: (1) Mirex: Samples of various fish and shellfish have been analyzed gas chromatographically for mirex contamination. Findings of some general interest include: (a) mirex "spikes" as generally used to estimate recovery are invalid, as they do not reach the internal pool; (b) mirex added in hexane to the outside of a sample or homogenate is recovered to a much

greater extent than is mirex added in DMSO when a "representative" sample of fish tissue can be taken for analysis by using a power driven cork borer to remove a plug extending through the body of the fish.

(2) Piperonyl butoxide: The purification of commercial piperonyl butoxide (the most important synthetic pesticidal synergist) as well as the characterization of its impurities has been achieved by column, TLC and GLC procedures. Among the impurities in technical grade material identified were: diethyleneglycol monobutyl ether; dihydrosafrole; 6-methyl dihydrosafrole; 6-propyl piperonyl methoxide; 6-propyl piperonal; 6-chloromethyldihydrosafrole; 6-propyl piperonylic alcohol; 2-propyl-4,5-dimethoxybenzyl-n-butyldiethyleneglycol ether; bis(2-propyl-4,5-methylenedioxyphenyl)methane; and di(2-propyl-4,5-methylenedioxybenzyl)ether. A number of these above components have been found to possess synergistic activity per se as well as to inhibit hepatic mixed function-oxidases.

(3) Polychlorinated biphenyls (PCB's): The quantitative and qualitative analysis of PCB's (1 to 3 chlorine atoms) by GLC and flame ionization as well as a method for predicting their retention indices was achieved. Solute-solvent interaction between these "non-polar" compounds and a variety of liquid phases was also elaborated.

(4) Heavy metals: The determination of total, organic and inorganic mercury in biological tissues has been accomplished employing atomic absorption, gas-liquid and thin-layer chromatography, respectively. Analogous procedures are being elaborated for the analysis of cadmium in biological tissues as well.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Branch as well as be of utility for other studies within the Institute.

PROPOSED COURSE: (a) To continue GLC analytical development for additional specific isomeric chlorobiphenyls and their metabolites; (b) To develop a GLC-MS analysis of the important mutagenic anti-schistosomal agent, hycanthone, in mouse lymphoma cells for investigations of the Mutagenesis Branch; (c) Phthalate Esters: Both TLC and programmed temperature GLC procedures are being elaborated for the separation and identification of the diester, half-ester, phthalic acid, 2-ethyl hexanol, 2-octanol and phthalic anhydride to both monitor the synthesis of purified di(2-ethylhexyl)- and dioctylphthalates as well as their potential metabolites.

PUBLICATIONS

Albro, P.W., Fishbein, L. and Fawkes, J.: Purification and characterization of pesticidal synergists. J. Chromatog. 65: 521, 532, 1972.

Fishbein, L.: Chromatographic and biological aspects of polychlorinated biphenyls. Chromatog. Revs. (In Press).

Fishbein, L. and Albro, P.W.: Chromatographic and biological aspects of phthalate esters. Chromatog. Revs. (In Press).

Serial No.: NIEHS-ASC-008
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Relationship Between Responsiveness of Calf Brain (Na^++K^+)-ATPase and Structural Characteristics of Inhibitory Pesticides, Fungicides and Drugs"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: W. E. Wilson, Ph.D.

OTHER INVESTIGATORS: C. W. Sharp; Ph.D.; N. K. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	3.0
Professional:	2.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The major objectives have been to ascertain the most probable mode(s) of action of chlorinated biphenyls, chlorinated hydrocarbon pesticides and several detergents as inhibitors of the beef brain microsomal (Na^++K^+)-ATPase system. This enzyme system is believed to be of fundamental importance in (a) control of mammalian cellular electrolyle balance, (b) maintenance of electrical potentials across nerve and other membranes, (c) transport of amino acids and (d) regulation of heart muscle contraction. Such an understanding may provide insight into other membranal control mechanisms which are related to, or are dependent upon, the energy transduction phenomena carried out by the (Na^++K^+)ATPase system.

METHODS EMPLOYED: Various spectrophotometric techniques, various chromatographic techniques, ultracentrifugation, automated enzyme analysis.

MAJOR FINDINGS: The enzyme analysis protocol has been changed from the automated technique for measurement of orthophosphate to a coupled enzyme system which measures ADP formation from ATP. The coupled enzyme system allows measurement of initial reaction velocities and, thereby, has permitted the unequivocal discrimination between protection against enzyme inhibition and reversal of enzyme inhibition. Pure phosphatidyl inositol (P.I., beef brain from General Biochemicals, Inc.) has been obtained which activates (Na^++K^+)-ATPase to about the same extent as a crude PI mixture which we had previously

isolated from beef brain. A number of chlorinated biphenyls have been studied and have been found to exhibit some interesting properties which appear to be intermediate between those of DDT and those of oleate or dodecyl sulfate. (1) Upon undergoing a phase change, individual PCBs exhibit spectral alterations similar to those noted for DDT in the uv region. (2) Aroclor 1221 differs from Aroclor 1254 or DDT in the ease with which it undergoes a phase change so that it no longer inhibits (Na^++K^+)ATPase activity. (3) CHs differ from detergents in that (Na^++K^+)ATPase inhibition by the latter exhibits distinctive temperature dependence. (4) (Na^++K^+)ATPase from beef and fish brain, or from rabbit kidney, are similarly responsive to DDT, Aroclor 1221 and Aroclor 1254. (5) Phosphatidyl serine (PS), phosphatidyl inositol (PI) and phosphatidyl choline (PC) provide protection against enzyme inhibition by anionic detergents or CHs; however, albumin provides good protection against anionic detergents but poor protection against DDT or Aroclor 1254. Phosphatidyl ethanolamine (PE) protection generally seems to be less effective than that by other phospholipids. (6) Except for PE, the phospholipids and albumin can reverse enzyme inhibition by anionic detergents. PS and PI are much more effective than PC, PE or albumin in reversing inhibition by DDT, Aroclor 1254 or Aroclor 1221. (7) Only PS and PI can protect against deoxycholate inhibition in our preincubation scheme. (8) On the assumption that the phospholipid selectivity for reversal of CH-inhibition of enzyme activity may have a common basis with observations by other investigators for phospholipid selectivity in activating phospholipase A treated (Na^++K^+)ATPase preparations, it can be hypothesized that CHs probably interfere specifically with phospholipid activation of the (Na^++K^+)ATPase. If we assume enzyme phospholipid complex (E·PL) can interact with inhibitor (I) as follows -

$$E\cdot PL + I \rightleftharpoons E\cdot PL\cdot I \rightleftharpoons E\cdot I + PL \text{ and/or } E + PL\cdot I,$$

then specificity for addition of phospholipids might be explained on the basis of specificity for phospholipids in the E·PL complex; i.e., the active enzyme. (9) Fatty acid profiles have been obtained for the various types of brain phospholipid; the degree of unsaturation is rather similar for PS, PE and PC (PI fatty acids need to be determined again).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated hydrocarbon pesticides are known to interfere with mammalian central nervous system function. The neuro-toxicity of DDT is claimed to result from interference with passive K^+ ion diffusion across the squid axion membrane; however, beef brain (Na^++K^+)ATPase inhibition appears to be slightly more sensitive to DDT than the various membranal preparations used in electro-physiological investigations on DDT toxicity. It is important to ascertain whether there is such a thing as a membranal weak link which would account for CH toxicity to mammals. (The recent studies by W. D. Kinter's group suggest that DDT and PCBs may facilitate lethal osmoregularity failure in several marine teleosts by specifically inhibiting several of their membranal (Na^++K^+)ATPase systems.) Since CHs can associate with various types of protein aromatic functional groups to significant extents, it may be expected that those membranal systems which are protein, in nature, will exhibit the most likely candidates as weak links. Our results showing phospholipid selectivity in reversing CH inhibition of the (Na^++K^+)ATPase may provide a handle in obtaining insight, at the molecular level, regarding the mode of action of the CHs as inhibitors of a model membranal system.

PROPOSED COURSE: Our observations of phospholipid selectivity for reversal of CH inhibition and for protection against deoxycholate suggest that a satisfactory general solution might be obtained to explain the mode of toxicity of DDT and of the PCBs. Several questions which can be asked include: (1) Can evidence from electron microscopy, light scattering or NMR studies (relaxation time studies using the C^{13} probe) of the phospholipids provide insight into properties which could account for selective inhibition reversal? (2) Do phospholipids preferentially bind Na^+ or K^+ as reflected by using the phosphorus probe in the NMR? (3) Do (Na^++K^+)ATPase or MgATPase systems from other species (fish, eels, rabbits and rats would represent a starting spectrum) and from various tissues (brain, kidney, gills [eel and fish] and intestine (eel or fish) show phospholipid selectivity for reversal of CH inhibition and/or protection against deoxycholate? (4) Can a technology be devised to purify the beef brain (Na^++K^+)ATPase?

PUBLICATIONS

Wilson, W. E., Clements, S. T. and Latimer, A.: Phosphatidyl serine protection of brain membranal (Na^++K^+)ATPase from inhibition by fatty acids and chlorinated hydrocarbon pesticide. Fed. Proc. 30(2): 2672, 1971.

Wilson, W. E. and Sharp, C. W.: Chlorinated biphenyl interaction with beef brain (Na^++K^+)ATPase. Fed. Proc. 31(2): 2119, 1972.

Serial No.: NIEHS-ASC-009
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism and Health Effects of Ingested Hydrocarbons"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate the relationship(s) of accumulation of dietary paraffins in animal tissues to such suspected pathologic effects as lipidoses, co-carcinogenesis and interference with reticular endothelial functions. (2) To determine the specificities of various animal tissues for the accumulation of individual hydrocarbons from defined diets. (3) To elucidate the pathway for catabolism of ingested hydrocarbons, particularly with respect to interference by drugs, pesticides and synergists.

METHODS EMPLOYED: The basic test organism is the rat, to which hydrocarbons are administered orally in corn oil, mixed with rat chow, injected I.P., I.V. or I.M. In some cases radioactive tracers (1-^{14}C-hexadecane, 1-^{14}C-octadecane, and U-^{14}C-phytane and 2,3-^{3}H-phytane synthesized here) are used in metabolic studies. Standard chromatographic, immunologic, radioassay and other biochemical techniques are used, except that balance studies of absorption from the gut are made as described in Biochim. Biophys. Acta. 219: 437-446, 1970.

MAJOR FINDINGS: (1) The hypersensitizing effect of dietary pristane toward anaphylactic shock in rats treated with ovalbumin has been found to be absent in splenectomized rats. Preliminary studies on the general effect are nearly ready for publication. (2) The major end product of phytane metabolism in rats is acetyl coenzyme A, with propionyl coenzyme A second. The pathway of degradation has not been established but is under study. Metabolism occurs mainly in the liver and kidneys. (3) Phytane is also oxidized in rat liver to 2,6,10,15-tetramethylhexadecan-2-ol, a tertiary alcohol. This alcohol accumulates, and its effects are presently unknown. Oxidation of these

isoprenoid hydrocarbons appears to be inducible both by phenobarbital and pristane, but quantitative in vivo studies are just begining.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ingestion of paraffins from a variety of substances and its accumulation (as well as that of other hydrocarbons) in human tissues in cases of lipoid pneumonia, follicular lipidosis of the spleen and meningioma all suggested that tissue accumulation of hydrocarbons is undesirable. Investigations are in progress to elaborate the parameters involved in access of hydrocarbons to the tissues. The most recent findings suggest a previously unsuspected pathway for oxidation of alkyl chains that may have general significance. A new metabolite has been identified which will be further studied with regard to elucidating its pharmacologic/physiologic effects.

PROPOSED COURSE: An attempt will be made to obtain sufficient 2,6,10,14-tetramethylhexadecan-2-ol and/or 2,6,10,14-tetramethylpentadecan-2-ol for feeding studies in order to determine whether they are implicated in the hypersensitization properties of pristane and whether detailed toxicological studies are likely to be fruitful. The study of the catabolic pathway for phytane will be continued, and in vitro studies will be emphasized in an attempt to identify the early intermediates of its breakdown. Technique will be refined in order to carry out more informative studies on the nature of the hypersensitizing properties of pristane.

Serial No.: NIEHS-ASC-014
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "NMR Investigations of the Stability of Metallic Linear and Cyclic Dithiocarbamates"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.25
Professional:	0.25
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the specific rate constant at various temperatures for the decomposition of a variety of fungicidal and related cyclic and linear metallodithiocarbamates, to calculate the activation energies and thermodynamic parameters for the process and to develop an experimental procedure to be adapted to similar studies on related compounds. (2) To determine the degree of partial double-bond character of the carbon-nitrogen bond (the barrier to rotation about this bond) in tetraalkyl thiuram sulfides.

METHODS EMPLOYED: Variable temperature proton magnetic resonance spectroscopy was employed.

MAJOR FINDINGS: The decomposition of ethyl selenac [tetrakis(N,N-diethyldithiocarbamato)selenium (IV)] was found to be kinetically first order both in ethyl selenac and in water. Major products are N,N,N',N'-tetraethyl thiuram disulfide and selenium. Intermediate products found, e.g., selenite and diethyldithiocarbamate, may also be of environmental significance. Activation thermodynamic parameters for the above decomposition were also elaborated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The cyclic and linear metallodithiocarbamates have wide utility as fungicides and rubber accelerators and, hence, are of environmental significance. Ethyl selenac has also been shown to be an effective enzyme inhibitor. Our studies regarding the kinetic elaboration of the decomposition of ethyl selenac have aided in the

elaboration of the decomposition products and rates of formation and, hence, may further illuminate both its mode of inhibition as well as the possible environmental hazard associated with its use.

PROPOSED COURSE: Project completed, February, 1972.

PUBLICATIONS

Wilson, N.K. and Fishbein, L.: Decomposition of tetrakis(N,N-diethyldithiocarbamato)selenium (IV). J. Agr. Food Chem. (In Press).

Serial No.: NIEHS-ASC-016
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Synthesis of Analogs and Possible Metabolites of Environmental Agents"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: L. Levy, Ph.D.

OTHER INVESTIGATORS: H. B. Matthews, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.6
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To prepare potential metabolites of saccharin (benzisothiazolone, S,S, dioxide), especially oxygenated derivative and N-hydroxy saccharin. To more fully investigate the chemistry of the parent nucleus about which relatively little is known compared with the isomeric benzthiazole series of compounds.

METHODS EMPLOYED: A variety of classical and modern synthetic techniques have been employed in this study. In some cases the appropriate synthetic methodology did not exist and had to be developed independently for the task at hand. The appropriate spectroscopic and chromatographic techniques were used to identify and analyze the various products obtained.

MAJOR FINDINGS: Of most significance has been the unique photochemical synthesis of N-alkoxy saccharin. This long-sought-after intermediate has exactly the skeleton and substitution suitable for conversion, by classical means, to N-hydroxysaccharin. However, this last step has not yet been completed successfully.

Last year's report dealt with the synthesis of N-methoxy dehydrosaccharin. This model made it possible to synthesize the corresponding N-benzyloxy derivative, analogous to the above-mentioned N-benzyloxy saccharin. Other ring-substituted saccharin derivatives have been prepared as possible metabolic products of saccharin. Because of difficulties in repeating some of the published procedures for the synthesis of several ring-substituted saccharin

derivatives, attempts to find new methods for creation of the heterocyclic part of the saccharin nucleus have been initiated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study has been initiated because of the paucity of knowledge concerning the chemistry and metabolism of saccharin, a chemical with extremely widespread usage as a food additive. Since N-hydroxylation has been shown to be a metabolic pathway for a number of environmental agents, e.g., cyclamate, anilino herbicides, etc., and since a variety of N-hydroxy (as well as N-nitroso) derivatives have also been shown to be carcinogenic, mutagenic and/or teratogenic, it is essential to elaborate the chemistry of such compounds as well as to extend the methodology available for their syntheses.

PROPOSED COURSE: The synthesis of saccharin derivatives will be limited to the preparation of N-hydroxysaccharin for further metabolic and mutagenic evaluation.

Serial No.: NIEHS-ASC-020
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Pesticide Binding Studies"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To develop methods for the fractionation of serum and tissue proteins that will allow isolation of purified proteins without displacement of bound pesticides. (2) To determine the types of binding interactions (covalent, hydrophobic, charge-transfer, π-interactions, etc.) involved in the biologically significant association of pesticides with proteins. (3) To determine whether a correlation can be made between binding forces and biological activity (structure-activity relationship).

METHODS EMPLOYED: Ion exchange chromatography, electrophoresis, "salting out", differential centrifugation and discontinuous density gradient centrifugation, and selective denaturation are used to fractionate blood previously equilibrated with DDT and its analogs, aldrin, etc. Pesticides are assayed by GLC and TLC and through the use of ^{14}C-labeled material.

MAJOR FINDINGS: Precipitation with Dextran-sulfate appears to be a suitable technique for purification of DDT-binding lipoprotein.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The relative toxicity of DDT and its analogs and metabolites, as well as other chlorinated hydrocarbons, cyclodienes and environmental agents such as the polychlorinated biphenyls, might be correlated with relative binding affinities of target protein or possibly with relative capacities of specific transport systems in the blood; hence, definitive information regarding the nature of the binding of these classes of environmental agents is of importance.

PROPOSED COURSE: Purified proteins will be compared in their ability to bind a spectrum of labeled and unlabeled agents including the isomeric DDT's, DDE and a number of individual isomeric chlorinated biphenyls, and the distribution of the above compounds among blood constituents will be examined in detail.

Serial No.: NIEHS-ASC-021
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Prostaglandins as Environmental Factors"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.3
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: (1) Development of a highly sensitive, specific analytical procedure for qualitative and quantitative determination of prostaglandins. (2) Evaluation as to whether exogenous prostaglandins should be considered environmental health factors, either relative to ingestion or topical exposure. (3) Determination as to whether endogenous prostaglandins play a significant role in the response of animals to drugs, pesticides, antigens and the like.

METHODS EMPLOYED: (1) A procedure has been developed by which prostaglandins may be assayed by gas chromatography (J. Chromatog. 44: 443, 1969). A two dimensional thin-layer chromatographic technique involving specific visualization reagents is under development for qualitative detection of prostaglandins. A procedure for extraction and purification of prostaglandins to be used as a preliminary to GLC and TLC has been developed which is suitable for screening purposes but which does not give adequate recoveries for quantitative assays. (2) A technique for studying the effects of prostaglandins on mast cells in vitro has been developed.

MAJOR FINDINGS: (1) Prostaglandins of the E type stabilize mast cells against spontaneous release of histamine, while A, B and F prostaglandins have no discernable effect. (2) Considerable difficulty has been experienced in obtaining mast cells from sensitized rats in order to test whether prostaglandin E also stabilizes against antigen-induced histamine release. Attempts are being made to use rabbit anti-egg albumin serum for passive sensitization to overcome some of the difficulties, but no results are available at the present time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because continuing review of the literature indicates that many of the properties of prostaglandins mentioned under "Objectives" above are currently under investigation in a number of laboratories, we have presently concentrated on the role of prostaglandins in the anaphylactic response as being most directly related to their possible environmental significance, but not presently under saturating study elsewhere. Our results to date suggest more of a protective than a harmful role for prostaglandins in anaphylaxis.

PROPOSED COURSE: (1) We still wish to make studies of the effects of orally administered prostaglandins on laboratory animals, but this will have to wait until gram quantities of prostaglandins become available. (2) We will complete our studies on the effects of prostaglandins or sensitized mast cells as soon as the technical difficulties in obtaining sensitized cells are overcome. A new technique for obtaining respiratory tissue mast cells appears promising (Science, 195: 1012, 1972), and will be considered for future use.

PUBLICATIONS

Albro, P.W. and Fishbein, L.: Prostaglandin: action of mast cells in vitro. Prostaglandins 1: 133-146, 1972.

Serial No.: NIEHS-ASC-022
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effect of Chronic Exposure in the Rat to Heavy Metals"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: M. D. Folsom, Ph.D.

OTHER INVESTIGATORS: J. Fawkes and A. Latimer

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	1.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the in vitro and in vivo effects of long-term exposure of the rat to small amounts of heavy metals by investigating storage and retention of the metals as correlated with effects on enzyme systems and other toxic effects.

METHODS EMPLOYED: Neutron activation analysis, atomic absorption, gas-liquid chromatography, thin-layer chromatography, enzyme assays of liver and kidney enzymes and histological studies have been utilized.

MAJOR FINDINGS: Methylmercury hydroxide, mercuric chloride and cadmium chloride and sulfate were administered to rats over periods of two weeks to three months. When methylmercury hydroxide at levels as high as 14 mg/kg was given over a two-week period, histological damage was observed in the liver and kidney. When the same total dose was given over a longer period (to 3 months), no damage was detected, suggesting the possibility of reversibility or repair of damage. Stored total mercury levels were comparable in both groups of animals, regardless of how the same total dose was administered. Small increases in hepatic microsomal cytochrome P-450 were noted. Other liver enzymes (e.g., nitroanisole demethylase aniline hydroxylase, β-glucuronidase and glucuronyl transferase) were not significantly affected. The same effect on kidney enzymes was noted. For example, alkaline and acid phosphatase, carbonic anhydrase and glucuronyl transferase were all inhibited. Total mercury levels in the kidney decreased more slowly compared to other tissues, e.g., liver, spleen, lung and muscle, after exposure to mercury was discontinued.

Urinary protein excretion was enhanced in animals receiving approximately 15 mg cadmium or methylmercury/kg.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is much concern over the possible effects of continued exposure to small amounts of heavy metals formed as contaminants in food, water and other environmental sources. Knowledge of the biochemical effects and storage mechanisms would be beneficial in determining the dimensions of the hazards of environmental exposure.

PROPOSED COURSE: Studies to further explore kidney involvement are in progress. Other enzyme systems will be studied (e.g., glucose-6-phosphatase, lactic dehydrogenase, glutamate dehydrogenase and glutamine alanine transaminase) as well as selected organocadmium compounds and the zinc cadmium interaction.

PUBLICATIONS

Folsom, M. and Fishbein, L.: Effects of repeated sub-lethal dosages of methylmercury in the rat. Science of the Total Environment. (In Press).

Serial No.: NIEHS-ASC-023
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Lung Mixed Function Oxidase Systems"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: M. Fields

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To study the mixed-function oxidase enzymes of mammalian lung and the effects of inhaled xenobiotics on these enzymes.

METHODS EMPLOYED: Ultracentrifugation techniques, enzyme assays, spectrophotometric assays and microsomal cytochromes and aerosol techniques have been utilized to test the effects of xenobiotics on the mixed-function oxidase (mfo) enzyme system.

MAJOR FINDINGS: The mixed-function oxidase enzymes of lungs are similar in many ways to the mixed-function oxidase enzymes of liver which have been studied extensively, the most notable exceptions being the techniques necessary for the isolation of these enzymes, the variability in activity from animal to animal and the lower yield of enzyme per gram of lung tissue. In vitro, the lung mixed-function oxidase enzymes are inhibited by insecticide synergists (I_{50} values are in the range of 10^{-5}M); and piperonyl butoxide, one of the insecticide synergists tested, has been shown to be metabolized by these enzymes. Also in vitro, these enzymes have been shown to be inhibited 15-25% when incubated under an atmosphere containing 0.1% carbon monoxide. Lung microsomal enzymes isolated from animals immediately after exposure to an aerosol of piperonyl butoxide for 15 min. are only 60 to 70% as active as similar preparations isolated from the lungs of unexposed animals. Still greater inhibition (up to 60%) is observed when animals are sacrificed and the enzymes isolated 15-24 hrs. after exposure. The enzymes are still not fully recovered at 48 hrs. after exposure.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pesticide synergists are widely used in aerosols formulations of insecticides which are used in the home as well as in dusts, sprays and impregnated liners. Large urban areas have daily peak concentrations of nearly 0.01% carbon monoxide and higher in restricted areas. If, as is probably the case, the mixed-function oxidase enzymes of the lungs serve to protect the lungs and consequently the individual from the effects of inhaled xenobiotics, inhibition of these enzymes by the contents of household aerosols and urban air may play a significant role in lowering the resistance of the exposed individual to the actions of any inhaled toxicant.

PROPOSED COURSE: (1) Continued work with xenobiotics which are known to inhibit the mfo enzyme (piperonyl butoxide and other pesticide synergists; CO and other gases). (2) Initiation of studies on the effects of combinations of xenobiotics on the lung mfo enzymes (insecticide synergists and CO; insecticide synergists and insecticides; and insecticide synergists and Freon gases).

Serial No.: NIEHS-ASC-024
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Dieldrin"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: J. D. McKinney, Ph.D.; M. Fields

COOPERATING UNITS: Cell Biology Branch

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To study the metabolism, storage and the rate of excretion of dieldrin in the rat following administration of doses, possibly analagous to that resulting from environmental exposure (e.g., a single 10 ppm oral admin.).

METHODS EMPLOYED: Thin-layer chromatography, liquid scintillation, NMR spectroscopy and mass spectral analysis.

MAJOR FINDINGS: Dieldrin is metabolized and excreted much more rapidly by male rats than by females. The major metabolite excreted by each sex is excreted in the feces. The major metabolite excreted in the urine by the males has been previously identified and designated "Kline's" metabolite. Female rats apparently do not excrete this dieldrin metabolite. Both sexes excrete an unknown water soluble metabolite in the urine and a small amount of aldrin-trans diol in both the urine and feces. Male rats excrete very little unmetabolized dieldrin (approx. 2%), whereas the females excrete approximately 25% of the dieldrin unmetabolized.

As expected, the primary site of dieldrin storage in each sex was the adipose tissue. In the tissues of the female and several tissues of the males, dieldrin was stored as almost 100% unmetabolized insecticide. In other tissues of the male, particularly the kidneys and lungs, a large portion of the stored insecticide existed as "Kline's" metabolite. The in vivo and in vitro systems shown to metabolize dieldrin in the above studies have been

used to provide Dr. McKinney with metabolites of dieldrin for his studies of the chemical nature of these compounds (NIEHS-ASC-032). Also the TLC systems developed in the above studies have been used to analyze the products of various chemical studies of dieldrin metabolites.

Aldrin-trans diol shown to be a metabolite of dieldrin in the above study, has been previously isolated in other studies; however, the cis isomer has not been previously isolated. We have demonstrated the metabolism of dieldrin to the cis diol and have shown that this cis diol is enzymatically converted to the trans diol. The "epimerization" has been confirmed by mass spectral analysis, gas chromatography, and thin-layer chromatography. Conversion of the cis to the trans is catalyzed by an enzyme(s) in the microsomal portion of a liver homogenate and requires NADPH as a cofactor; NADH can also serve as a cofactor, but less efficiently, and NADPH and HAD are ineffective as cofactors.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dieldrin is a "hard" pesticide and even though its use is declining, its existence in the environment will continue for some time; therefore, definitive knowledge of its metabolism is essential.

PROPOSED COURSE: The elaboration of the toxicity of the dieldrin metabolites as well as the effects of multiple administration of small doses of dieldrin in the rat will be undertaken. We also hope to isolate enough of the cis diol to trans diol conversion product to compare its optical rotation with that of the trans diol isolated as a metabolite of dieldrin by other workers.

PUBLICATIONS

Matthews, H.B., McKinney, J.D. and Lucier, G.: Dieldrin metabolism, excretion and storage in male and female rats. J. Ag. Food Chem. 19(6): 1244, 1971.

McKinney, J.D., Matthews, H.B. and Fishbein, L.: The major fecal metabolite in the rat. Its structure and chemistry. J. Ag. Food Chem. (In Press).

Serial No.: NIEHS-ASC-025
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Mirex in the Rat and Uptake in Plants"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: H. B. Matthews, Ph.D.; L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To study (1) metabolism, storage and excretion of a dose of mirex such as might be encountered from air environmental exposure; (2) metabolism of mirex by various liver preparations from rat, mouse and rabbit; (3) metabolism of mirex by plant root preparations; and (4) uptake by plants.

METHODS EMPLOYED: Chromatographic (paper, TLC, GLC, column), liquid scintillation and radioautography.

MAJOR FINDINGS: At the end of 7 days, following a single oral dose of mirex-^{14}C (6 mg/kg) in the rat, 58.5% of the dose was excreted in the feces, less than 1% in the urine, with the rest stored in the body. No metabolite of mirex was detected in feces, tissues or urine. Excretion in the feces of mirex was rather rapid in the first two days. Mirex was not metabolized by any rat or mouse liver preparation when incubated with various cofactors and for prolonged periods up to 36 hours. Pea and bean root preparations known to be biochemically active also failed to metabolize mirex.

Pea and bean plants take up mirex from water with 1, 5 and 10 ppm levels, which are quite likely to be encountered within the areas of continued mirex application. Roots concentrated mirex (6.5 and 9.5 ppm at 1 ppm and 71.3 and 101.6 ppm at 10 ppm, in peas and beans, respectively) to sizeable levels. After high concentrations (7 to 10 times the concentration in water) are stored in the roots, a small amount is translocated to the aerial parts.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mirex is now becoming well recognized as a pesticide which is highly resistant to degradation. No evidence of its biodegradation has been reported. Since the compound is advocated in the Southeast for large scale fire-ant control, information as to its stability, metabolism and persistence is of importance.

PROPOSED COURSE: The study has been completed and in view of other investigations in progress in other laboratories, the project will be terminated at this stage.

PUBLICATIONS

Mehendale, H.M., Matthews, H.B. and Fishbein, L.: Fate of mirex in the male rat and uptake in plants. Bull. of Environmental Contam. & Toxicol. (In Preparation).

Serial No.: NIEHS-ASC-027
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Complex Equilibria Involving DDT"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None.

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To determine the thermodynamic parameters, e.g., interaction energies, for formation of complexes between the pesticide, p,p'-DDT and model compounds of biological interest.

METHODS EMPLOYED: Variable temperature proton magnetic resonance spectroscopy.

MAJOR FINDINGS: Two types of complexes were observed, one involving electron donation by the complexing agent into the DDT aromatic π-electron system, and the other an electrostatic interaction between a polar complexing agent and the highly polar DDT benzhydryltrichloromethyl system. Binding energies were found to be greatest between DDT and aromatic donors and between DDT and organic phosphate groups. This suggests that DDT may interact *in vivo* primarily at aromatic sites in protein or at phosphate linkages.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: DDT's ubiquitous distribution and known deleterious effects on living organisms give importance to characterization of its potential interactions.

PROPOSED COURSE: Project completed, October 1971.

PUBLICATIONS

Wilson, N. K.: Complex equilibria involving 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane. The NMR spectrum and types of complexation. *J. Amer.*

Chem. Soc. (In Press).

Wilson, N. K. and Wilson, W. E.: Complex equilibria involving DDT and model compounds for phospholipid and protein. Biochem. Biophys. Research Commun. (Submitted for Publication).

Serial No.: NIEHS-ASC-028
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Saccharin"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; A. Latimer; M. Fields

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.3
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the in vitro and in vivo metabolism of saccharin and derivatives.

METHODS EMPLOYED: Variety of chromatographic (column, paper TLC, GLC) and radioautographic techniques.

MAJOR FINDINGS: When fed a dose equivalent to a normal human exposure (1 mg/kg/day) for seven days, both male and female rats rapidly excrete saccharin, primarily in the urine, without any apparent biological alteration of the molecule. The rapid excretion of saccharin accounts for the fact that an insignificant amount of this compound is stored in any of eleven tissues and organs investigated. In vitro studies with preparations from several organs have also failed to yield a metabolite of saccharin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolism of this important food additive has not been fully elucidated despite its extensive utilization for many decades. The possibility of potential harmful metabolites of saccharin, e.g., N-hydroxy and related derivatives, cannot be ruled out.

PROPOSED COURSE: To investigate the possible role of intestinal microbiological activity on saccharin stability.

Serial No.: NIEHS-ASC-029
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Substituted Amphetamines and Tertiary Amino Propiophenones by Monoamine Oxidases"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: V. S. Bangdiwala, Ph.D.

OTHER INVESTIGATORS: B. Corbett, E. O. Oswald, Ph.D., and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.5
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: Monoamine oxidases are enzymatic systems—localized in brain, liver, kidney, blood and many other tissues—which are responsible for the oxidative deamination of biologically important amines, producing ammonia plus the corresponding carbonyl compound. Monoamine oxidase inhibitors bring about the accumulation of serotonin, tyramine and other amines in the brain. This excessive accumulation of biogenic amines may produce abnormal neurological responses of the individual.

Injection of substituted allyl benzene derivatives of essential oils has resulted in psychoactive responses. Early postulations surmized that these components of the essential oils were converted to amphetamines. The postulated amphetamine would then elicit the psychoactive responses. More recently we have isolated and verified that substituted allyl benzenes of the essential oils are converted biologically to nitrogen-containing tertiary amino propiophenones.

Using monoamine oxidase preparations, the effect produced by the postulated amphetamine and the documented metabolite of the allyl benzene, e.g., the tertiary amino propiophenones, on this important biological system will be elaborated.

METHODS EMPLOYED: U.V., visible spectroscopy, cellular fractionation by ultracentrifugation and monometric assays; I.R., N.M.R. and mass spectrometry; and

thin-layer, column and GLC chromatography were utilized.

MAJOR FINDINGS: Following oral or intraperitoneal administration of myristicin, safrole, elemicin and other allyl benzenes to rats and guinea pigs, these animals initially are highly active and excited. During the initial 15-30 minutes after administration of these propenyl benzene derivatives, the animals are hyperactive. After about 30 minutes, the animals in the highly excited state become very sedated, immobile and non-responsive to sound and motion for periods up to two hours. Myristicin, safrole, isosafrole, asarone (trans), beta-asarone (cis), elemicin, eugenol and other propenyl benzene derivatives appear to produce a stimulant and then a tranquilizing effect.

Guinea pig liver contains a monoamine oxidase system that is about one and one-half times as high as in rat liver using tyramine or serotonin as substrates. In addition, the guinea pig liver monoamine oxidase system is much easier solubilized than the rat liver enzyme. However, with benzylamine as substrate, rat liver preserves approximately four times as much active monoamine oxidase as does guinea pig liver. In addition, rat heart contains about four times more monoamine oxidase than does guinea pig heart.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough understanding of the interaction of the tertiary amino propiophenone and various amphetamines with monoamine oxidase is essential in the overall understanding of the psychoactive properties of the component of essential oil and in the evaluation of these environmental agents as potential hazards.

PROPOSED COURSE: Complete inhibition studies with amphetamines and tertiary amino propiophenones using crude mitochondrial preparations. Solubilize and purify the most desirable monoamine oxidase system. Compare inhibition studies upon purification with those of the crude enzyme sizes. Isolate and identify metabolites of the tertiary amino propiophenones found during reactions.

PUBLICATIONS

Oswald, E. O., Fishbein, L., Corbett, B. J. and Walker, M.P.: Urinary excretion of tertiary amino methoxy methylenedioxy propiophenones as metabolites of myristicin in the rat and guinea pig. Biochem. et Biophys. Acta 244: 322-328, 1971.

Serial No.: NIEHS-ASC-030
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Organic Syntheses of Radioactive Labeled Propenyl Benzene Derivatives"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: B. Corbett; M. P. Walker; L. Fishbein, Ph.D.; and J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.3
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Substituted allyl and propenyl benzene derivatives are widely distributed in nature. In order to determine whether these environmental agents constitute a potential hazard, investigations are in progress to isolate, quantitate, and identify the various metabolites of myristicin, safrole, isosafrole, asarone, beta asarone, and dihydrosafrole following administration by various routes to the rat and guinea pig.

METHODS EMPLOYED: Organic syntheses, chromatography (TLC, column and GLC), I.R., N.M.R., and mass spectroscopy.

MAJOR FINDINGS: The major problem in the syntheses of these radioactive labeled materials has not been the reaction sequence concerned with the labeling process, but instead has been the reaction sequence used to obtain the desired unlabeled precursors. Most types of reactions used for the precursors will not afford a pure component. The earlier methods yield complex mixtures of isomeric compounds. Attempts to prepare hydroxyeugenol, the precursor for myristicin syntheses, using Fremy salt were unsuccessful. This reaction is highly variable and conversion is not always to the same compound. We have prepared, however, hydroxyeugenol by the allylic rearrangement in a good yield and with a chemical purity of about 98%.

All of the reactions for the conversion of hydroxyeugenol to myristicin

encompass the conversion of the labeled iodoform to methylene iodide which is then condensed with the unlabeled hydroxyeugenol to yield myristicin labeled in the methylenedioxy ring. The above reaction conditions afford an approximate 40-50% yield of the unlabeled myristicin from iodoform.

^{14}C Methylenedioxy labeled myristicin was prepared on a five millimolar scale from ^{14}C iodoform in a 56% yield with a radiopurity of 98% with specific activity of 0.78 mc/mMole. In addition, using the five millimolar scale, ^{14}C-methylenedioxy labeled safrole was prepared in a 29% yield with radiopurity of 99% and a specific activity of 0.68 mc/mMole.

Serial No.: NIEHS-ASC-031
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Biosynthesis of Piperidine and Pyrrolidine"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: J. D. Peele, D. Gregg, J. Phillips, B. J. Corbett and L. Fishbein, Ph.D.

COOPERATING UNITS: Pathologic Physiology Branch

MAN YEARS

Total:	1.5
Professional:	0.2
Other:	1.3

PROJECT DESCRIPTION

OBJECTIVES: Allyl benzene metabolic derivatives are linked biologically to tertiary amino propiophenones, e.g., the amine condensing units of each ketone are dimethylamine, piperidine and pyrrolidine. Very little information is available on the biosynthesis of these particular secondary amines. It is very desirable to determine the biological sequence of events that yield dimethylamine, and especially piperidine and pyrrolidine. Piperidine and pyrrolidine possess "nicotine-like" properties in biological systems. In addition, the correlation of psychoactive properties of allyl benzene metabolites of essential oil with that of these secondary amines should enhance the evaluation of potential hazard of these materials as environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), cell fractionation by ultracentrifugation of enzyme analyses and radioactive tracer metabolism followed by N.M.R., I.R. and mass spectrometry.

MAJOR FINDINGS: Preliminary composition enzyme analyses using Dopa as substrate for the decarboxylase in rat and guinea pig liver, kidney, brain and intestine indicate that the decarboxylase system under a nitrogen atmosphere requires pyridoxal phosphate for measurable activity. Under the conditions of analysis at pH 6.8 only about 20% of the enzymatically released carbon dioxide is bound; in addition, using 0.01M Dopa as substrate, the enzyme activity is linear for at least twenty-five minutes. Rat and guinea pig liver, kidney, brain and intestine contain significant measurable amounts of Dopa decarboxylase. Guinea

pig kidney is the most active source of Dopa decarboxylase (200-400 µl CO_2/1 hr/200 mg tissue) while rat intestine is the least active tissue containing Dopa decarboxylase (50-75 µl CO_2/1 hr/200 mg tissue).

At the present time, measurable decarboxylase activity with proline and pipecolic acid varies from 0-15 µl CO_2/1 hr/200 mg tissue. Because of the very low decarboxylase activity, additional investigations are underway to investigate the products of piperidine and pyrrolidine from ^{14}C labeled proline and pipecolic acid.

Derivatization of piperidine and pyrrolidine with 2,4-dinitrofluorobenzene followed by extraction with cyclohexane yields very satisfactory materials for spectrophotometric and chromatographic analysis of these secondary amines. These highly fluorescent derivatives can be quantitated spectrophotometrically at 350 mµ with linearity from 0 to at least 5.0 µg/ml. In addition, the dinitrophenyl derivatives of piperidine and pyrrolidine are separable by thin-layer chromatography on silica gel GF using cyclohexane:butyl acetate (80:30).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding and knowledge of the rates of production and elimination of piperidine and pyrrolidine are essential in determining the potential of these biological materials as health hazards.

PROPOSED COURSE: Characterize and purify the decarboxylase systems responsible for the conversion of proline and pipecolic acid to pyrrolidine and piperidine, respectively. Characterize the formation of piperidine and pyrrolidine from ^{14}C labeled glutamic acid and ^{14}C labeled lysine using the rat, guinea pig and some level of primates. Investigate the bacterial decarboxylase responsible for formation of piperidine and pyrrolidine.

Serial No.: NIEHS-ASC-032
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Dieldrin and Related Polycyclodiene Pesticides as Model Systems for Chemical Study of Enzyme Reaction Mechanisms - (A) Oxygenation, (B) Epimerization"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: H. B. Matthews, Ph.D.; H. Mehendale, Ph.D. and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To extend our knowledge of certain enzyme reaction mechanisms facilitated by the unique chemical structure and biological stability of dieldrin and other related chlorinated polycyclodiene pesticides, their metabolites and derivatives. To establish the utility of these systems as model compounds for the study of differential binding properties and metabolism of certain steroid hormones and their precursors.

METHODS EMPLOYED: A variety of synthetic, chromatographic, autoradiographic, and spectroscopic techniques, particularly high resolution proton magnetic resonance (PMR) spectroscopy.

MAJOR FINDINGS: (A) Our studies have dealt primarily with the major metabolites of dieldrin excreted by the rat. The stereochemical structure (C-12 syn-hydroxydieldrin) has been established by PMR analysis according to methodology previously developed in our laboratory. The major fecal metabolite could not be converted *in vitro* to the major urinary metabolite; however, a common cationoid intermediate, analogous to that proposed in aromatic hydroxylation, may exist in their formation from dieldrin. Female rats do not excrete the major metabolite isolated from male rat urine. In this regard, there is a closer similarity between the stereochemical behavior of dieldrin and its major metabolites and certain male steroid sex hormones and their precursors.

In separate studies involving in vitro metabolism of aldrin by plant root extracts, cis and trans-diols as well as mono-hydroxy compounds are formed from pathways other than aldrin epoxidation followed by epoxide hydration. (B) Cis-hydration of epoxides is in some ways a more stereochemically favorable process than trans-hydration. The chemical ring opening of the epoxide group in dieldrin under "enzyme like" conditions (intimate ion pair formation) afford cis-products in good yields. In vitro metabolism studies of dieldrin have indeed shown the presence of the cis-diol. In addition, the cis diol does undergo in vitro inversion of configuration at carbon (epimerization) to produce the trans-diol and may account for the optical activity observed for this metabolite of dieldrin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated polycyclodiene pesticides are among the most stable and persistent insecticides that contaminate our environment. Their continued use necessitates the elucidation of their metabolic pathways in mammalian systems. A great deal of insight has been obtained via the study of bridged polycyclic compounds such as dieldrin. Perhaps greater significance will be derived from the use of these systems as model compounds for studying enzyme reaction mechanisms as well. It is possible that such studies will enable one to design compounds which may be of therapeutic use, e.g., all of the enzymatic reactions being investigated for dieldrin and aldrin have their analogies in steroid metabolism.

PROPOSED COURSE: To investigate a possible biological antagonism between dieldrin systems and certain male or female hormones and to delineate the role of metabolite conjugates in dieldrin metabolism. To elucidate the intermediates involved in the cis-diol to trans-diol epimerization reaction and to establish cis-hydration as a significant process in the hydration of certain sterically constrained epoxides such as dieldrin.

PUBLICATIONS

McKinney, J.D., Matthews, H.B. and Fishbein, L.: Major fecal metabolite of dieldrin in rat. Structure and Chemistry. J. Agr. Food Chem. 20: 3, 1972.

Matthews, H.B., McKinney, J.D. and Lucier, G.W.: Dieldrin metabolism, excretion and storage in male and female rats. J. Agr. Food Chem. 19: 1244, 1971.

Keith, L.H., Alford, A.L. and McKinney, J.D.: NMR studies of the chlorinated polycyclodiene pesticides. Analytical Chimica Acta. (In Press).

Serial No.: NIEHS-ASC-033
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Chemistry of Hydroxydichlorobenzhydryl Type Metabolites of DDT, Their Precursors and Degradation Products"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; J. E. Suggs, Ph.D.

COOPERATING UNITS: Toxicology Branch, Environmental Protection Agency, Chamblee, Georgia

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the unique chemical behavior of the hydroxydichlorobenzhydryl type metabolites and delineate their biological role; and to illuminate the nature of DDT-metabolite conjugates which are not extractable into organic solvents and do not appear to be the usual conjugates of glucuronic and sulfuric acids.

METHODS EMPLOYED: A variety of synthetic, chromatographic and spectroscopic techniques.

MAJOR FINDINGS: The unique chemical behavior of these systems can for the most part be attributed to the nature of the carbon-containing moiety attached to the benzhydryl carbon, e.g., the trichloromethyl group of kelthane. These DDT metabolites and their conjugates in certain cases can function as one-carbon donors with strong oxidizing ability and, in other cases, as alkylating agents. In all cases, byproducts of chemical reactions are known DDT metabolites, many being of the unsaturated type such as DDE. The formation of hydroxydichlorobenzhydryl systems from their benzhydryl precursors appears to be both chemically and biologically feasible.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A knowledge of the theoretical chemistry of DDT should provide a basis for studying the interactions of DDT in biological systems on a molecular level,

i.e., chemical interactions, charge transfer complexation, etc., such that metabolism as well as storage propensities can be better understood. The chemistry revealed thus far is consistent with the unusual behavior observed for the DDT-metabolite conjugates as well as with the possible oxidative involvement of such hydroxy analogs with reduced porphyrin systems.

PROPOSED COURSE: To investigate the interaction products of the hydroxy metabolites with biological entities both *in vitro* and *in vivo*.

PUBLICATIONS

McKinney, J. D., Hawk, R. E., Boozer, E. L. and Suggs, J. E.: On the chemistry of the isomeric dichlorodiphenylacetaldehydes related to DDT. Stereoelectronic effects in the hydrolysis of their cyclic acetal. *Can. J. Chem.* 49: 3877, 1971.

McKinney, J. D. and Fishbein, L.: DDE formation. Dehydrochlorination or dehypochlorination. *Chemosphere*, March, 1972.

Serial No.: NIEHS-ASC-034
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Biphenyls, Chemical Prototypes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: P. Chen, Ph.D. and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.3
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To develop chemical prototypes for comparison with enzymatic hydroxylation of biphenyls in order to reveal the electronic nature of the enzymatic reaction, i.e., one electron or two electron oxidation. To provide small quantities of synthetical metabolites for analytical comparison with authentic metabolites.

METHODS EMPLOYED: A variety of synthetic, chromatographic and spectroscopic techniques.

MAJOR FINDINGS: The chemical ionic electrophilic substitution model for studying the enzymatic hydroxylation of biphenyl and 4-chlorobiphenyl afforded the major in vitro metabolite of these compounds in the rat. Other in vivo metabolites can be explained on the basis of cationoid and epoxide intermediates. The chemical radical electrophilic substitution model cannot be ruled out, especially in regard to the formation of o-dihydroxy metabolites via peroxide intermediates. The ionic reaction does provide a synthetic route for small quantities of the suspected epoxide intermediates. The ionic reaction is totally inhibited by the presence of competitive (1 to 1) amounts of sulfhydryl containing compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is necessary to isolate, quantitate, and identify the metabolites and other conversion products of the PCB's to ascertain whether or not exposure or

ingestion of these agents represent a potential environmental hazard. An understanding of the chemical reactions involved in the metabolism of these compounds should facilitate the localization, isolation and characterization of their isolable and intermediary metabolites.

PROPOSED COURSE: To investigate the interactions and subsequent reactions of the biologically important, proposed epoxide intermediates with various biological entities. To note differential behavior in the metabolism of biphenyls and the usual benzenoid systems. To determine any differences in metabolisms and toxicities of the more highly substituted polychlorinated biphenyls due to stereochemical factors.

Serial No.: NIEHS-ASC-035
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Hexachlorobenzene and Hexachlorocyclopentadiene"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: H. B. Matthews, Ph.D.; R. L. Metcalf, Ph.D.; L. Fishbein, Ph.D.

COOPERATING UNITS: University of Illinois

MAN YEARS

Total:	0.05
Professional:	0.05
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the pharmaco-kinetics of hexachlorobenzene and hexachlorocyclopentadiene in male and female rats, the in vivo and in vitro metabolism of the two compounds in the rat, the metabolism and fate of the two compounds in a model ecosystem, and the effect of the compounds on the overall biochemistry of the animals' liver using rats as an experimental animal.

METHODS EMPLOYED: A single radioactive dose of the compound such as might be encountered in the environment will be administered to both sexes of rats. Metabolism, excretion and storage will be studied. In addition, in vitro metabolism by liver preparations from rats, mice and rabbits will be investigated.

The model ecosystem will include an interaction of terrestrial and water interphases as follows: (1) the terrestrial interphase will include corn plants on which the compounds will be applied; (2) insect caterpillars which feed on the plants will be present; (3) insect excreta will introduce compounds into the water phase of the model ecosystem; and (4) a food chain consisting of snails, water crustaceans, mosquito larvae, algae and fish will be exposed to the above toxicants. The metabolic fate and bioaccumulation of the toxic agents will be studied at the organismic level and at the food chain as a whole.

MAJOR FINDINGS: The project has just been initiated as a cooperative project between the Institute and Dr. R. L. Metcalf of the University of Illinois.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hexachlorobenzene is a common fungicide used as a seed-dressing to protect seeds from various fungal diseases. It is known to cause porphyria cutanea tarda in human beings, characterized by extreme sensitivity to sunlight. The effects can last beyond cessation of ingestion of the compound. An understanding of the metabolism, storage and excretion pattern would be helpful in understanding the mechanism of the induction of porphyria. Elaboration of the metabolism of hexachlorocyclopentadiene (an intermediate in the production of mirex) is desired since it would be likely to be another environmental contaminant introduced along with mirex.

PROPOSED COURSE: The project has just been initiated. In vivo and in vitro metabolism, pharmaco-kinetics and induction studies will be carried out at the Institute. Studies involving the model ecosystem will be conducted on a cooperative basis with Dr. R. L. Metcalf of the University of Illinois.

Serial No.: NIEHS-ASC-036
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Nuclear Magnetic Resonance Studies of Substituted Biphenyls"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None .

COOPERATING UNITS: None

MAN YEARS

Total:	0.25
Professional:	0.25
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To study the detailed molecular and electronic structure of several substituted biphenyls and their possible interactions with complexing agents which may be of biological importance.

METHODS EMPLOYED: Variable temperature NMR and supportive computor methodology.

MAJOR FINDINGS: The relationships between NMR parameters and the calculated (CNDO/2) electron densities in several p,p'-disubstituted biphenyls have been found.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should help to illuminate the types of interactions in which the substituted biphenyls can participate. The polychlorinated biphenyls, in particular, are of wide current interest as environmental hazards. This work should increase our understanding of how they may be biologically active.

PROPOSED COURSE: This work will continue, and will be extended to include biphenyls substituted at other positions and with a greater degree of substitution.

Serial No.: NIEHS-ASC-037
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "In vitro Metabolism of PCB"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. R. Chen, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To study the mode of action and metabolic fate of a variety of isomeric PCB's.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), N.M.R. and mass spectroscopy.

MAJOR FINDINGS: Rat liver microsomes fortified with NADPH can metabolize various mono- and dichloro-substituted biphenyls. The major metabolite of the monodichloro biphenyl was identified as the 4-hydroxyl compound. These products have been well characterized by TLC and I.R., N.M.R. They had identical R_f values and spectra as the authentic compounds. With dichloro-substituted biphenyl, rat liver microsomes fortified with NADPH only hydroxylated those compounds where 4' position was opened.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the biotransformation and the identification of the metabolites of the ubiquitous and environmentally important PCB's is essential in understanding their mode of action and potential hazard to environmental health.

PROPOSED COURSE: Quantitative aspects of metabolism will be investigated when ^{14}C-radioactive PCB's are available. The possibility of an epoxide being the intermediate of this hydroxylation will be investigated since epoxides of aromatic hydrocarbons are active biologically. The metabolic fate in the soil will be investigated because it is one of the three major reservoirs of these environmental agents.

Serial No.: NIEHS-ASC-038
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Induction of Hepatic Microsomal Enzymes by Three Tetrachlorobiphenyls"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. R. Chen, Ph.D.

OTHER INVESTIGATORS: H. Mehendale, Ph.D. and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the biochemical effects of several isomeric tetrachlorobiphenyls in the rat.

METHODS EMPLOYED: Enzyme assay, thin-layer chromatography, radioautography.

MAJOR FINDINGS: No growth retardation and enlargement of liver in rats were found following oral administration of three tetrachlorobiphenyls (2,3,4,5-; 3,5,3',5'-; 2,4,2',4'-tetrachlorobiphenyls) at three different levels. The activities of four drug metabolizing enzyme systems were measured. Aniline hydroxylase activty and ethyl morphine demethylase activity were elevated 1.5 fold at the dose level of 20 mg/kg, but p-nitroanisole demethylase activity was enhanced 2-fold at the lowest dose (5 mg/kg). In general, the response of these enzyme systems to the PCB studied were higher in male animals. UDPG-glucuronyl transferase was not affected by these environmental agents.

The in vitro metabolism of 17 β-oestradiol was studied in pretreated female animals. Radiochromatograms revealed that an additional polar metabolite was formed (which was dose-dependent).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elaboration of the in vitro and in vivo metabolism of specific purified isomers of the polychlorinated biphenyls is essential to the assessment of the hazard of this important class of ubiquitous environmental agents.

PROPOSED COURSE: Studies of PCB-induced hepatic porphyria will be carried out via an examination of δ-aminolevulinic acid synthetase activity in the rat following the administration of a number of isomeric chlorinated biphenyls.

Serial No.: NIEHS-ASC-039
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Identification and Characterization of Environmental Agents by Mass Spectrometry"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: P. W. Albro, Ph.D.; J. D. McKinney, Ph.D. and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	1.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Our present computerized Mass Spectrometry facilities, including the Finnigan 1015 Electron Impact system and the Finnigan 1015 Chemical Ionization unit afford the capability of collecting and correlating a tremendous amount of spectral information. Because chemical ionization mass spectrometry is most useful in direct compliment with electron impact data, all of the compounds are analyzed and correlated on both systems. This catalogued information will serve as an identification library of various organic environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column, and GLC), I.R., N.M.R. and mass spectrometry, computer analyses, organic syntheses.

MAJOR FINDINGS: The present Finnigan 1015 - electron impact and chemical ionization units have decreased sample requirements by a factor of 1,000 - 10,000 fold over the older Perkin-Elmer 270 unit. In addition, because both Finnigan units are controlled by the computer, all of the data from initiation up to the end of a particular analysis are stored on magnetic tapes. The system 150 computer makes data available for analyses within minutes after collection. The routine sample size for most environmental agents under investigation is a maximum of one microgram. (Sample sizes of 50-100 nanograms will also yield very informative spectra).

The newer chemical ionization unit has the same sensitivity requirements as that of the electron impact unit; however, the quasi molecular ion (parent +1) or in some cases (parent -1) is much more intense in the former case. In addition, chemical ionization spectra in most cases are much less complex, leading to a much simpler interpretation.

Comparing the tertiary amino propiophenones of elemicin on the electron impact and chemical ionization systems, under normal operating conditions of 70 EV with the electron impact unit and separation of these materials by GLC, the parent ion varying in mass from 269-309 is present in only 0.2-0.4% abundance relative to the base peak. However, using the chemical ionization system, ring spectra of these same elemicin metabolites contain the quasi molecular ion (parent +1) in a 2-15% abundance, depending upon various conditions. In general, there is at least a 10-fold and in some cases up to 100-fold increased intensity of the molecular ion in chemical ionization mass spectrometry.

Additional advantages of the chemical ionization spectra are seen in investigations concerning branch alkane hydrocarbon and alcohol of these specific hydrocarbons.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A detailed collection of electron impact and chemical ionization mass spectra which can be retrieved with the speed of a computer will serve as a characterization and identification library of the various potential environmental hazards.

PROPOSED COURSE: Environmental agents to be characterized include: A. Pesticides (dieldrin - aldrin derivatives; polychlorinated materials; herbicides; carbamates and phosphates). B. Natural Products (prostaglandins and other lipids; hydrocarbons; essential oil components and metabolites; amines and amino acids; toxins and flavoring agents). C. Medicinals (amphetamines; phenols and tertiary amino propiophenones). D. Miscellaneous (organic mercury, nickel and cadmium compounds; food additives and components of plasticizers and/or plastics, e.g., phthalate esters).

During the characterization and identification of the desired environmental agents, mass spectra which have been collected by the computerized system will be obtained for both electron impact and chemical ionization. In addition, the chemical ionization mass spectrometer will be used to study various gas phase reactions involved in specific organic syntheses.

Upon purchase of the magnetic disc system, a library with the pertinent mass fragments of each compound will be initiated for identification and characterization of the various environmental agents and their metabolites.

PUBLICATIONS

Oswald, E.O., Fishbein, L. and Corbett, B.J.: Metabolism of naturally occurring propenyl benzene derivatives. II. Separation of tertiary amino propiophenones by gas-liquid chromatography. (In preparation for submission to J. Chromatog.).

Serial No.: NIEHS-ASC-040
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Purification of the Beef Brain $(Na^+ + K^+)$ATPase System"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: C. W. Sharp, Ph.D.

OTHER INVESTIGATORS: W. E. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To obtain a pure preparation of beef brain $(Na^+ + K^+)$ATPase to ascertain whether this enzyme requires specific phospholipids for normal stabilization. Such knowledge would be imperative for a true understanding of phospholipid selectivity in reversing chlorinated hydrocarbon (CH) inhibition of this enzyme.

METHODS EMPLOYED: Spectrophotometry, ultracentrifugation, polyacrylamide electrophoresis, radioactive tracer techniques, cryogenic techniques, molecular distillation and various chromatographic techniques.

MAJOR FINDINGS: The $(Na^+ + K^+)$ATPase is inhibited by DDT and a number of the PCBs. Inhibition may be reversed by specific phospholipids. Because the phospholipids are pure and because the selectivity in phospholipid reversal of PCB or PCB inhibition cannot be rationalized on the basis of preferential interaction of phospholipid with inhibitor, such selectivity must reflect one or more properties of the enzyme. The enzyme has been purified to the extent of 25-50% by Hokin's group. We believe that technology can be developed to achieve more extensive purification of the enzyme. Our experience at this time suggests that we may be able to take advantage of the apparent stabilizing effect of phosphatidyl serine to effect further enzyme purification.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At present, there are very few membranal enzyme systems which have been truly purified. At the present time it is also difficult to rationalize biological

effects of environmental agents such as CHs at the molecular level because appropriately responsive membranal components are not available for study. Thus, an effort is required to attempt to obtain such purified components.

PROPOSED COURSE: An attempt will be made to design supports which would permit final enzyme purification according to the principle of affinity chromatography. The supports will contain structural analogs of ATP or of phosphatidyl serine. This will initially involve several synthesis problems. Analogous techniques are known for synthesis of a support containing an analog of ATP; however, developmental time is required to work out the design for the support containing a structural analog of phosphatidyl serine.

An attempt will be made to determine whether the membranal lipid enzyme activity might be reversed by solvent extraction (at low temperatures) and then added back to reconstitute enzyme activity. By adding back specific phospholipids it may be possible to demonstrate reconstitution of enzyme activity with concomitant alteration in the properties of the reconstituted microsomal material which could be conducive to easier purification of the enzyme.

Serial No.: NIEHS-ASC-041
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Aldrin Metabolism by Plant Root Preparations"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To improve methods of studying in vitro metabolism in plant tissue preparations and to study the metabolism of aldrin by plant root preparations.

METHODS EMPLOYED: Various chromatographic techniques (TLC,GLC,column); centrifugation.

MAJOR FINDINGS: Aldrin epoxidase activity in pea and bean root preparations was enhanced considerably by including p-aminobenzoic acid (10^{-4} to 10^{-5}M) in the buffer and for both extraction and incubation. The demonstrated increase in activity was over and above that already manifested by use of polyvinyl pyrrolidone during homogenization of roots.

More efficient and purified preparations could be obtained by homogenization rather than by using root soaking method for extracting aldrin epoxidase from plant roots. Almost all the activity in the cell-free preparations obtained by root soaking could be ascribed to the 250,000 x g pellet. The possibility that there are two or more enzyme systems acting on aldrin was borne out by the results obtained when various preparations were incubated in the presence of NADPH. In several respects these plant preparations are similar to animal liver enzymes.

Dormant seeds or early germinates do not have aldrin epoxidase activity. Onset of the enzyme activity occurs on the 5th and 6th day in peas and beans, respectively, when the hypocotyls are about 1/2 inch or longer.

Dieldrin is the major metabolite of aldrin in these plant in in vitro reactions. Other minor metabolites detected were cis- and trans-aldrin diols. These diols were detected only when aldrin was incubated and did not form when incubations were made with dieldrin as substrate. These observations, coupled with the detection of both isomers of aldrin diol, indicate that aldrin undergoes biotransformation through two distinctly different pathways.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aldrin is a major chlorinated cyclodiene pesticide and is a common environmental contaminant. While its fate in almost all target and nontarget organisms is fairly well understood, that in plants has not been fully elaborated. The nature of the residues one will encounter in the environment depends partly on the metabolic transformation that aldrin undergoes in plants. Thus, an understanding of the pathways of metabolism of pesticides to which environmental agents of this nature may be subjected in plants would be valuable. Demonstrating and refining in vitro techniques for plants for studying the degradation of pesticides is an important asset in understanding the environmental hazards of pesticide residues.

PROPOSED COURSE: Mass spectroscopic evidence for the presence of cis- and trans-diols will be obtained. Efforts will be directed toward developing in vitro techniques for demonstration of heptachlor epoxidation in plants.

PUBLICATIONS

Mehendale, H.M.: Aldrin epoxidation by plant root preparations. (Submitted to Pesticide Science, 1972).

Mehendale, H.M. and McKinney, J.D.: Identification of cis- and trans-aldrin diols as metabolites of aldrin by plant root preparation. (In Preparation).

Serial No.: NIEHS-ASC-042
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effect of Feeding Mirex on Rat Liver and Its Microsomal Enzymes"

PERVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. M. Mehendale

OTHER INVESTIGATORS: P. R. Chen, Ph.D.; H. B. Matthews, Ph.D.; L. Fishbein, Ph. Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To study the effect of orally administered daily doses of mirex (e.g., 10, 25 and 50 mg/kg mirex in corn oil) on the male and female rat liver and liver microsomal enzymes.

METHODS EMPLOYED: Spectrophotometric elaboration of the following enzyme activities: (a) N-demethylation of ethyl morphine, (b) O-demethylation of p-nitroanisole, (c) aromatic hydroxylation of aniline, and (d) glucuronidation of 1-naphthol.

MAJOR FINDINGS: While normal body weight gain was reduced significantly in both male and female rats at 10, 25 and 50 mg/kg treatments, in females it was much more drastic. At 25 and 50 mg/kg dosages, female rats actually lost weight, Although total administered mirex was considerably less than that required for acute oral toxicity, some female rats at 25 and 50 mg/kg died after 3 to 5 days. None of the male rats treated at similar levels died. Gross pathological examination of these rats revealed no pathological observations ascribable in the cause of death.

Livers were enlared enormously and, although generally increase in size of the liver was in proportion to the dosage, most of the increase in size was manifested at 10 mg/kg and only smaller degrees of further increase occurred at higher levels. Liver to body weight ratio was doubled in both male and female rats (4.37 to 8.59 and 4.63 to 9.42 at 10 and 50 mg/kg levels in males and females (respectively).

Ethyl morphine N-demethylation: While both male and female liver enzyme activities were induced, that in males was not progressive with dose. At 50 mg/kg levels there was actually a decrease in the enzyme activity. In the females, all three dose levels resulted in an increase in activity which was at its peak at 25 mg/kg. Activity in female rats was induced to a higher degree than in males.

P-nitroanisol O-demethylation: Enzyme induction occured at 10 mg/kg in both sexes. Higher levels caused a decrease in activity in male; in females increased activity was reduced at 25 mg/kg and was less than in controls at 50 mg/kg. Induction in the females was greater than in the male.

Aniline hydroxylation: All three treatments caused a reduction in aniline hydroxylation in both males and females. The decrease in activity was dose dependent. This reduction in activity is not due to the physical presence of mirex per se, because amounts from 25 mg to 2 mg were without any effect on the enzyme activity in vitro.

The actual amount of mirex residue in the microsomal enzyme preparations was determined by concentrated GLC (10 to 37 ug/incubation at 10 to 50 mg/kg respectively). The addition of mirex to incubations at levels up to 60 times that found in the treated liver was without effect on in vitro aniline hydroxylation. It is possible that 10 mg/kg, the lowest dose in these experiments, was in excess of that required to observe induction. However, the possibility that mirex might act to inhibit the activity in the animal liver might not be ruled out.

Glucuronidation of 1-naphthol: Generally, there was no effect on glucuronyl transferase.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Mirex is one of the most persistent pesticides known yet and will be undoubtedly ingested by man as a result of environmental exposure. An evaluation of its effect on the general overall biochemistry of the liver via an examination of hepatic microsomal activity is desirable in order to more fully understand the implications of its hazard as an environmental pollutant.

PROPOSED COURSE: The inverse relationship of induction, inhibition and extreme sensitivity of aniline hydroxylase to mirex will be pursued further to gain some understanding of the cause-effect relationship of mirex. Measurements of P-450 will be included in such studies.

PUBLICATIONS

Mehendale, H.M., Matthews, H.B. and Fishbein, L.: Induction of rat liver microsomals in the male and female rats by mirex. (In Preparation).

Serial No.: NIEHS-ASC-043
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Syntheses of Labeled and Unlabeled Polychlorinated Biphenyls (PCBs)"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: L. Levy, Ph.D.; M. P. Walker and J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: (1) Gas chromatography - Mass spectroscopy: Preliminary investigation has been undertaken to determine the major components of the 1221, 1242, 1254 and 1260 Aroclors. Available standards and synthesized materials were used to verify the major components of these mixtures. Once sufficient synthetic, pure compounds are available and the major components are verified, biological investigations will be carried out with the various pure individual major components of the series 1200 Aroclors.

(2) Organic Syntheses: Individual, pure standard components of the mono-, di-, tri-, tetra-, penta-, hexa-, hepta- and octa-chlorinated derivatives of the Aroclors are being synthesized from commercially available materials. In addition, ^{14}C-labeled chlorinated species in the pure form are being synthesized for a variety of metabolic and photolytic studies as well as for intra-branch investigations.

(3) Metabolism: Radioactive ^{14}C-labeled major components of the series 1200 Aroclors will be used to determine tissue distribution, excretion and structural modifications that may occur in the rat and other mammalian systems.

METHODS EMPLOYED: Organic syntheses, chromatography (TLC, column and GLC), I.R., N.M.R. and Mass Spectroscopy, Radioactive monitoring and quantitation.

MAJOR FINDINGS: Most of the organic reactions available for preparation of the

polychlorinated biphenyls result in low yields and/or a complex isomer mixture. The most useful reaction is the Gomberg Reaction utilizing diazotization of the substituted aniline and coupling either with benzene or some other acceptor. In addition, there is modified Gomberg in which the aniline is converted to the acylarylnitrosamine which is coupled with the appropriate acceptor. Coupling of iodo-chlorobenzene with copper using the Ulmann reaction is also possible; however, in general, the yield is very low. Use of the Ulmann reaction for highly chlorinated species is also difficult, in some cases hydrogenolysis predominates over coupling.

At the present time we have prepared biphenyl, the three mono-chlors, seven dichlor-, two trichloro- and six tetrachlorobiphenyls as pure standard materials. In addition, we are preparing on a 2-millimole scale the following labeled materials: ^{14}biphenyls, ^{14}C-2chlorobiphenyls, ^{14}C-3 chlorobiphenyl and ^{14}C-4-chlorobiphenyl with specific activities of about one millicurie per millimole in a yield of 40-60% from the ^{14}C-ring labeled substituted aniline derivatives.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCBs are known to be complex mixtures and are recognized to be of major environmental importance due to their ubiquitous ecological distribution and concentration in the food chain. Availability of purified labeled and unlabeled isomeric chlorinated biphenyls would permit more definitive diverse biological and toxicological studies and, hence, allow a more logical delineation of their potential hazard.

PROPOSED COURSE: Prepare additional labeled and unlabeled di-, tri-, penta-, hexa-, hepta- and octachlorobiphenyls.

Serial No.: NIEHS-ASC-044
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Utility of Rare Earth Metals in the Analysis of Environmental Chemical Hazards"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To test the previously observed complexing ability of complexes of the rare earth metals, europium and praseodymium, for selective complexation of impurities in the analysis of diverse environmental agents and their metabolites from various biological fluids and tissues.

METHODS EMPLOYED: A variety of synthetic chromatographic and spectroscopic techniques, in particular electron impact and chemical ionization mass spectrometry, are being used.

MAJOR FINDINGS: The utility of europium complexes in conjunction with proton magnetic resonance for enabling rapid assessment of stereochemistry in the metabolites of the chlorinated polycyclodiene pesticides has been clearly established. Europium nitrate has been used as a selective complexing agent of impurities in the determination of residue levels of the hydroxylic metabolites of the chlorinated polycyclodiene pesticides via their trimethylsilyl derivatives.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Selective complexation of impurities in biological extracts would allow rapid detection of much lower concentrations of metabolites without laborious prior clean-up procedures.

PROPOSED COURSE: To seek more specifically selective complexing agents which can be applied to various body fluids and tissues, etc., to facilitate isolation and characterization of metabolites.

PUBLICATIONS

McKinney, J.D., Fishbein, L. and Barling, L.: Selective complexation of impurities in the EC-GC determination of some chlorinated polycyclodiene pesticide metabolites and derivatives. Bull. Environ. Contam. Toxicol. 7(1): 2, 1972.

Serial No.: NIEHS-ASC-045
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Polychlorinated Biphenyls"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D.; L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the mammalian metabolism of labeled and unlabeled isomeric chlorobiphenyls.

METHODS EMPLOYED: A variety of chromatographic and mass spectroscopic techniques.

MAJOR FINDINGS: Chlorinated biphenyls having from one to six chlorine atoms per molecule were fed to rats at doses between 5 and 100 mg/kg body weight. Less than 10% of the amounts fed were excreted in the feces, indicating a high degree of absorption and/or metabolism of these compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Unequivocal identification of the metabolites and their tissue distribution, as well as knowledge of the rates of absorption and elimination of the isomeric polychlorinated biphenyls are essential for the assessment of the potential hazard of these ubiquitous environmental agents.

PROPOSED COURSE: Identification and characterization of the metabolites of a selected number of labeled and unlabeled isomeric chlorobiphenyls; and elaboration of their rates of absorption and elimination following oral and i.p. administration to the rat.

PUBLICATIONS

Albro, P.W. and Fishbein, L.: Intestinal absorption of polychlorinated biphenyls in rats. Bull. Env. Contam. Toxicol. (In Press).

Serial No.: NIEHS-ASC-046
1. Analytical & Synthetic Chemistry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Transport and Metabolism of Phthalate Esters"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; R. Thomas

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To prepare purified standard labeled and unlabeled phthalate esters, e.g., DHP [di(2-ethylhexyl)phthalate] and DOP (dioctyl phthalates); and to elaborate aspects of their transport, absorption, excretion and tissue residues following various routes of administration, e.g., oral, inhalation and i.p., in the rat.

METHODS EMPLOYED: Chromatography (TLC, column, GLC), spectrophotometry (I.R., U.V., visible), mass spectrometry and isotopic methods.

MAJOR FINDINGS: The gross non-homogeneity of commercial samples of DHP and DOP have necessitated the direct synthesis of pure materials for standards. TLC has been used to separate the diester, half ester, phthalic acid, 2-ethyl hexanol and phthalic anhydride enabling the monitoring of the synthetic process.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PROPOSED COURSE: Development of programmed temperature GLC of phthalate esters and their major metabolites, synthesis of ^{14}C-labeled DHP and DOP, and elaboration of mammalian metabolism of DHP and DOP.

ANIMAL SCIENCE AND TECHNOLOGY BRANCH

ANIMAL SCIENCE AND TECHNOLOGY BRANCH
Summary Statement

The Animal Science and Technology Branch functions in 3 program areas: Comparative Biology and Medicine, Animal Procurement and Husbandry, and Veterinary Services. All contribute to the Branch goals which are:

1. Procurement of disease-free animals of appropriate sex, age, strain and species, housed in a stable programmed environment, allowing NIEHS research to be conducted with a biological system exposed to a minimum of uncontrolled variables.

2. Development and refinement of animal models that are the most expedient tool with which to conduct specific research programs.

3. Environmental health research appropriate to the function of Branch laboratories; specifically, Hematology and Clinical Chemistry, Comparative Biology, Clinical Medicine, Diagnostic Pathology and Microbiology.

During the past year, the support activities of the Branch have been conducted at a level more consistent with Institute requirements. Major factors responsible for the increased support capabilities are occupancy of new animal facilities, addition of scientists who provide expert direction to laboratory activities and increased competence of animal care personnel. The essential segments of the Branch are formed, and efforts are now focused upon refining and broadening programs in a manner consistent with attainment of total operational capacity.

Facilities

Occupancy of Building 15 early in the Fiscal Year increased net animal holding facilities from 3,554 square feet to 16,187 square feet. The Institute now has a building of sophisticated design in which research animals are maintained in a physically and biologically controlled environment.

Building 2 under went modest renovation and now has cage washing equipment, a necropsy area and small rooms which may accommodate studies with radioactive or volatile compounds.

Personnel

Extensive training was provided in the function and proper operation of a barrier operated animal facility. Much of this training was incorporated into the continuing inservice training sessions which deals with general biology and fundamentals of laboratory animal care.

A cooperative training program with the Durham based New Careers Office was completed. The objective was to train 6 individuals, selected from financially, educationally or culturally deprived backgrounds, in order to qualify them for permanent positions as Biological Aides within the Animal Science and Technology Branch. In addition to training in areas specific to their jobs, general education classes leading to attaining a high school equivalency certificate were an integral part of the program. The proportion of candidates successfully completing the program was disappointing. A revised curriculum is being formulated after a thorough evaluation of the initial program. Another cooperative program is scheduled to commence late in the fiscal year.

Classes demonstrating basic techniques of handling laboratory animals were held for scientists and technicians. The training session, which is to be offered on an annual basis, emphasized humane procedures and proper methods of sample administration and collection.

The delay, and in some cases inability to promote lower grade employees created serious morale problems. Management's credibility with these employees has been damaged. Continuation of grade restrictions, due in part to grade reduction mandates, will make future efforts in promoting and teaching upward mobility or career ladder programs a complete mockery.

Program

Support Activities

It has been this Branch's goal to more actively involve animal care personnel in learning experimental procedures. This would provide animal care personnel an interesting and rewarding work experience and at the same time utilize Institute personnel in duties more appropriate with grade level. Consistent with this policy, 3 breeding colonies of animals are supervised and operated within the Animal Care Section, feeding studies are conducted and oral or parenteral dosing is routinely performed.

Disease surveillance and quality control programs are effected through the Clinical Medicine, Diagnostic Pathology and Microbiology Laboratories. All animal shipments are monitored with over 1,000 animals necropsied and processed according to protocols appropriate to the species. As a result of these programs, the quality of research animal used at NIEHS has improved. Chronic experiments are now conducted without fear of premature termination due to intercurrent infection.

Since activation of the Hematology and Clinical Chemistry Laboratory, about 40% of its efforts have been devoted to the processing of specimens submitted by Institute scientists. This laboratory allows experiments to be evaluated in a continuing manner without having to solely resort to endpoint histopathological analyses.

Research Activities

Research involving the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin dealt with further characterization of its teratogenicity and toxicity. Using the C57Bl/6 mouse, the specific period of fetal development which is susceptible to TCDD was investigated. Extension of these studies to the postnatal period in order to more effectively assess its action on nephrogenesis revealed that neonates develop polycystic kidneys. This is only the second example of a fetal chemical exposure resulting in tubular dysgenesis. Adult oral toxicity experiments indicate early and progressive effects on liver function. This study will hopefully indicate avenues of further research leading to an understanding of the mechanism of action of this extremely toxic chemical.

Several studies dealing with the effects of 3 purified polychlorinated biphenyls are in progress. The pathological, immunological, hematological and clinical chemical parameters are being investigated. Tissues are being analyzed within the Analytical and Synthetic Chemistry Branch which seeks to define the tissue distribution. There appears to be a positive correlation between the severity of the toxicity and increased chlorine content of the biphenyl. The interrelationship of an environmental contaminant and an infectious disease is being investigated utilizing various dose levels of PCBs on mice inoculated with Moloney leukemia virus. Studies on the effect mobilization of fat stores may have on animals which have received chronic doses of PCBs is also planned.

A disease due to the protozoan parasite Encephalitozoon cuniculi continues to interfere with interpretation of brain and kidney lesions seen in the rabbit species. In a cooperative effort with scientists at Ohio State University, ASTB has commenced a program designed to characterize the transmission and pathogenesis of the disease. These experiments, coupled with refinement of a skin test recently developed by OSU, will result in procedures being developed leading to eradication of the disease.

A study of the effect of household detergents on the rabbit eye was prompted by changes in product formulations. It was dramatically shown that some phosphate substitutes have severe corrosive properties and accidental introduction to the eye could produce impaired vision.

Several projects were concerned with the effects of heavy metal salts on the host defense mechanism. Immune capability was depressed as assessed by humoral antibody or interferon production after exposure to salts of lead, cadmium, arsenic or mercury in the drinking water. Direct challenge with lethal concentrations of virus gave variable results depending upon the system employed.

Hycanthone methane sulfonate, a drug used in schistosomiasis therapy, was examined for teratogenic potential following several reports citing it as a mutagen. Mouse studies found it a potent teratogen; once again depicting a

correlation between a mutagen and teratogen.

Evaluation of the captive-born opossum as a research model continued. These animals continue to remain parasite-free in our system of housing. Improved management practices produced a marked decrease in post weaning mortality and deaths due to endocarditis. Preweaning mortality remains high. Second generation animals had a conception percentage approaching 70%. First generation females retained through a second breeding season had very poor reproduction, suggesting that opossums should not be retained for a second year at this time. Hematological and clinical chemistry profiles are being developed now that stable, disease-free animals of known age and background are available. Cutaneous papillomas were found on a captive-born opossum. Electronphotomicrographs revealed virus particles which resemble the virus associated with this neoplasm in other species.

Serial No.: NIEHS-AST-001
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Disease Surveillance of Laboratory Animals at NIEHS"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: Loren D. Koller, D.V.M., Ph.D.
John A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	2.5
Professional:	1.0
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: To screen incoming, resident and alternative source laboratory animals for spontaneous infectious diseases and parasites.

METHODS EMPLOYED: All animals are examined for ectoparasites and endoparasites. A fecal flotation method is employed for detection of helminths and coccidia. Standard microbiologic laboratory techniques are employed. Precise regimen is determined by the particular species.

A. Rats, Mice and Hamsters:

1. Selective media for isolation of Salmonella.
2. Culture of lung and nasal washings for isolation of bacterial respiratory pathogens and Mycoplasma.
3. Viral Serology
   Mouse - Reo 3, GDVII, Sendai, K. Polyoma, LCM, MHV and Mad.
   Rat - Sendai, Kilham Rat and PVM.
   Hamster - PVM, SV5, Sendai and LCM.

B. Guinea Pigs:

1. Culture of lung washings for respiratory pathogens.

C. Rabbits:

1. Culture of lung washings for respiratory pathogens.
2. Serologic detection of Rabbit Venereal Spirochaetosis (Treponema cuniculi) employing the Rapid Plasma Reagin and VDRL tests.

D. Culture of particular organs or fluids from above species plus opossums and galagos, that have died spontaneously, as indicated by necropsy findings.

MAJOR FINDINGS: A. Rats, mice and hamsters have been free of Salmonella. Respiratory pathogens such as Mycoplasma pulmonis and Pasteurella pneumotropica were occasionally isolated from rats, especially those in residence 1 month or more. Two colonies of C57Bl/6 mice have been eliminated because of enzootic Pasteurellosis. Several rodent sources have been cancelled because of ecto-parasites and endoparasites.

Significant titers of Sendai and GDVII are in most groups of mice received. Titers to Sendai are also prevalent in rats. One group of mice had relatively high titers for polyoma.

B. A new source for guinea pigs was chosen because older sources were infected with the respiratory pathogen, Streptococcus zooepidemicus. Subsequent animals have been free of infectious disease.

C. New Zealand White and Dutch Belted rabbits, both incoming and resident, are infected to varying degrees with Pasteurella multocida. Approximately 75% of the infections are subclinical; the remainder may show overt respiratory disease, especially if stressed. The majority of rabbits are infected with intestinal coccidiosis; hepatic coccidiosis is rare. Persistent diarrhea, suspected to be of Escherichia coli origin, has caused intermittent fatalities among NZW rabbits. One source of Dutch Belted rabbits has a 10% incidence of venereal spirochaetosis. These animals are culled and the progency are not used.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is a continued effort to provide biomedical research with laboratory animals free of infectious diseases. It is equally important to determine which diseases animals may have been exposed to and which lesions are associated with that disease. This data allows one to eliminate diseased animals from research and should help preclude the chance of misinterpreting data due to unknown intercurrent infection.

PROPOSED COURSE: Surveillance of laboratory animals will continue on a routine basis.

Serial No.: NIEHS-AST-006-3
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Helminth Parasites of the Virginia Opossum (Didelphis virginiana)"

PREVIOUS SERIAL NUMBER: NIEHS-AST-006-2

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To identify helminth parasites infecting wild-captive opossums and to determine what lesions (if any) are inflicted upon the host. To compare the parasites infecting offspring weaned in captivity with the helminths infecting their mother.

METHODS EMPLOYED: Standard techniques were employed for collection, fixation and preservation of specimens. Identification of parasites was through original published descriptions and from comparison of specimens at the U. S. National Museum Helminth Collection, U. S. Department of Agriculture, Beltsville, Maryland.

MAJOR FINDINGS: A total of 17 helminth species were identified from 211 necropsies; 14 of these species inhabited the gastrointestinal tract. No deaths could be attributed to parasitism. Four helminth species caused obvious destruction of host tissue. Two of these species, Acanthocephala sp. and Paragonimus kellicotti, were rarely found and are not considered to be significant. Ninety-two percent of all opossums examined had pulmonary lesions associated with infestation by the lungworm, Capillaria aerophila. The pathogenic response was judged never to be severe enough to significantly impair lung function. The most significantly destructive parasite was the stomach worm, Physaloptera turgida, which caused focal necrosis of the gastric mucosa. Perforation of the stomach wall was not observed in this study, but is known to occur. Massive Physaloptera infestation conceivably could occlude the pylorus.

Opossums weaned in captivity at 3 months of age were free of all parasites, except for transient unidentified larvae which were no longer discovered in animals older than 5 months of age. (See NIEHS-AST-013).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is a descriptive and visual guide to identification of opossum parasites and is intended to aid investigators utilizing this animal. Although most of the parasites are innocuous, elimination or at least drastic reduction of the "parasite load" will concomitantly reduce extraneous variables in experimental data. Production and maintenance of young in vermin-free premises interrupts the life cycle of the parasites thus a parasite-free colony is easily attained.

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Feldman, D. B., Moore, J. A., Harris, M. W. and Self, J. L.: Characteristics of common helminths of the Virginia opossum (*Didelphis virginiana*) from North Carolina. *Lab. Animal Sci.* 22: 183-189, 1972.

Serial No.: NIEHS-AST-007-3
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: NIEHS-AST-007-2

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D.
John A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	1.15
Professional:	0.60
Other:	0.55

PROJECT DESCRIPTION

OBJECTIVES: Identify the sequency of toxicologic processes which develop following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

METHODS EMPLOYED: Seven to eight week old rats will receive 0.1, 1.0 and 10 µg/Kg oral doses of TCDD daily for 30 days. On days 3, 10, 17, 24 and 31, rats will be killed, blood taken for hematologic and clinical chemical determinations and a complete necropsy performed with tissues taken for subsequent histopathologic study. In addition, liver function will be studied by use of hexobarbital sleeping times and BSP clearance. Plasma and tissue porphyrins and liver δ-amino-levulinic acid synthetase (ALA-S) levels will be determined.

MAJOR FINDINGS: Early data evaluation indicates that rats receiving 10 µg/Kg TCDD have impaired liver function as evidenced by increased hexobarbital sleeping time, decreased BSP clearance and increased transaminase levels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data pertaining to TCDD toxicologic properties and the mechanism by which it produces its toxic effect are requisite for rational evaluation of its role as a health hazard.

PROPOSED COURSE: Evaluate the results of the present study. Develop protocol for a similar study using very low doses over a prolonged period in order to

study clinical pathologic and other biologic effects. Further work on the mechanism of impaired liver function.

Serial No.: NIEHS-AST-013-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Anthelmintic Treatment of Opossums in a Breeding Colony"

PREVIOUS SERIAL NUMBER: NIEHS-AST-013

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To evaluate a series of anthelmintics, singly and in combination, against the common opossum helminths in the attempt to eliminate as many parasites as possible without subjecting the animal to repeated treatment.

METHODS EMPLOYED: Six anthelmintics were evaluated: carbon disulfide-piperazine complex, dichlorvos, drocarbil, l-tetramisole, thiabendazole and thiabendazole-piperazine complex. Combinations of the above were subsequently administered.

Experimental animals were necropsied 7-14 days post-treatment. The number of parasites remaining in the gastrointestinal tract and lungs was tabulated.

MAJOR FINDINGS: No anthelmintic employed affected the lungworm, Capillaria aerophila. The CS_2-piperazine complex was effective in eliminating the stomach worm, Physaloptera turgida, and significantly reducing the oxyurid population. Trematode numbers were reduced with dichlorvos, but the trichostronglyle population was unaffected. In contrast, l-tetramisole produced the opposite effect.

Combining piperazine-CS_2 complex with either dichlorvos or l-tetramisole affected maximal overall reduction of gastrointestinal helminths.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study demonstrated the difficulty in eliminating all internal parasites from

wild-captive opossums by employing anthelmintics. These drugs are too parasite specific to affect the entire diverse population infesting the opossum. Previous studies have revealed that opossums weaned in captivity were parasite-free by the age of 5 months. It is apparent that parasite-free opossums can be realized through establishment of a captive-born closed colony.

PROPOSED COURSE: This study has been completed. A manuscript is being prepared for publication.

Serial No.: NIEHS-AST-014-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Evaluation of a Captive-Born and Reared Colony of Opossums"

PREVIOUS SERIAL NUMBER: NIEHS-AST-014

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the physiologic, pathologic and reproductive parameters of a "closed" colony of captive-born opossums.

METHODS EMPLOYED: Captive-born opossums were weaned at approximately 4 months of age and were housed in a separate quarantine building. Animals were reared individually in a screened cage provided with a nesting box. The diet consisted of 3 parts dry cat chow moistened with 1 part ground bovine liver. No dietary supplements were added. Water was provided ad libitum.

Animals were periodically examined for internal parasites and Salmonellae through routine fecal examination. All moribund animals were euthanatized and necropsied. With the onset of the breeding season, partitions separating a female and male opossum were removed and the animals permitted to breed at will. Records of births were maintained.

MAJOR FINDINGS: During the first year of this study, high mortality was experienced which was attributable to 3 factors: (1) Weaning at too young an age; (2) Bacterial vegetative endocarditis; (3) Death of males due to fighting of breeding pairs. During the current year, selected individuals from the previous year have been retained and the colony expanded to accommodate second generation captive-born opossums.

There has been a marked reduction in mortality of opossums due to a regimen of weaning at 4 months rather than at 3 months and a decrease in deaths attributable

to vegetative endocarditis. Male mortality, due to fighting during the breeding season, continues to be high. The conception rate among second generation animals is 70% as compared to 39% for first generation opossums bred in 1971. First generation opossums retained for 2 consecutive breeding seasons have a significantly lower conception rate (25%) with fewer young born per litter. Fifty per cent of pouch young die prior to weaning. Factors accounting for this high "fetal" wastage have yet to be defined.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The second year of this study further indicates the feasibility of maintaining a closed or quarantined nucleus of captive opossums for breeding purposes. Further improvement and refinement of techniques associated with the use of opossums suggests that they are a fit physiologic model on which to conduct more sophisticated types of research.

PROPOSED COURSE: Maintenance, enlargement and continued monitoring of closed colony.

Serial No.: NIEHS-AST-015
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Hematologic and Clinical Chemical Parameters in the Normal Virginia Opossum (Didelphis virginiana)"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: Donald B. Feldman, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To determine hematologic and clinical chemical parameters of normal opossum, especially with respect to age and sex.

METHODS EMPLOYED: The opossum samples will be obtained from a closed colony at NIEHS. Standard methods of hematology and clinical chemistry will be used.

MAJOR FINDINGS: It is too early to report any findings.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the use of opossums in research is increasing, it is imperative that researchers have methods of assuring themselves that their animals are healthy as well as methods of evaluating experimental procedures on the health of these animals.

PROPOSED COURSE: To continue the project in an effort to compile hematologic and clinical chemical profiles of normal caged opossums, especially with respect to age and sex of the animals.

Serial No.: NIEHS-AST-016
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Cutaneous Papillomas on an Opossum"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.10
Professional:	0.05
Other:	0.05

PROJECT DESCRIPTION

OBJECTIVES: To identify cutaneous papillomas in a new species of animal and to determine if the papilloma was caused by a papova virus.

METHODS EMPLOYED: Part of the cutaneous papilloma was surgically removed from the lateral aspect of the left front foot of a 1-1/2 year old opossum born and raised in captivity. The warts had been present for approximately 8 months. A 20 to 30 percent suspension in physiological saline solution was prepared by grinding the papilloma in a Ten Brok grinder. The papilloma suspension was negatively stained with 2 percent phosphotungstic acid and examined with the electron microscope.

The virus suspension was tested in agar gel for antigen. The center well was filled with antigen, and the outer wells contained serum collected at the time of removal of the papilloma and 1-1/2 months later. Fifteen captured opossums with an estimated age range from 3 to 8 months were inoculated with opossum papilloma suspension that contained 10,000 units of buffered potassium penicillin G and 10 mg of streptomycin sulfate U.S.P. per one ml of inoculum. The virus suspension was rubbed into scarified areas of skin, and 0.1 ml was inoculated intradermally with 0.5 ml papilloma suspension on the dorsal right foot.

Human papilloma virus was inoculated similarly on the opossums left thorax and on the foot of two. Warts from four different humans were utilized. Three were from the hand and one from the foot (planter type).

The opossum with original papillomas was inoculated in a similar manner as the other experimental animals 1-1/2 months after partial removal of the natural occurring papillomas.

Pieces of the papilloma were fixed in 10 percent buffered formalin or gluteraldehyde for histological and electron microscopic examination.

MAJOR FINDINGS: Cutaneous papillomas were on the left front foot and leg as well as the ear flap of a 1-1/2 year old opossum. The opossum was born and raised in captivity. The papillomas began to regress eight months after first noticed and had almost disappeared by 1 year. The warts occurred as papillomatous projections. They had a hyperplastic stratified squamous epithilum and a connective tissue base and core.

Virus particles resembling those in the papova group were identified in negative stained suspensions when examined by electron microscopy. The virions were approximately 50 mμ in diameter.

Virus containing suspensions from the opossum and different human papillomas did not produce papillomas in other opossums when inoculated intradermally or rubbed into scarified skin.

It is suggested that the opossum papillomas may have originated from exposure to human papillomas that were on the hand of an animal caretaker.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because papillomas have not been previously reported in opossums and the disease occurred in an opossum born and raised at the Institute, it is possible the papilloma originated from human papilloma virus. The animal caretaker had two small papillomas on the back of his hand before the papillomas were first noticed on the opossum. Perhaps this is an oncogenic virus that is transmissible from man to opossum.

PROPOSED COURSE: Neonate opossums in the mother's pouch will be inoculated with bovine, human and opossum papilloma virus in an attempt to experimentally produce papillomas on the opossum.

PUBLICATIONS

Koller, L. D.: Cutaneous papillomas on an opossum. Journal of the National Cancer Institute. (In Press)

Serial No.: NIEHS-AST-017
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Quantitation of Bovine Papilloma Virus by the Radial Immunodiffusion Test"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.10
Professional:	0.05
Other:	0.05

PROJECT DESCRIPTION

OBJECTIVES: To develop a method to quantitate the bovine papilloma virus. No methods are now available.

METHODS EMPLOYED: Bovine papilloma virus partially purified and then concentrated by ultracentrifugation is used as an antigen. Antiserum is obtained from calves with subcutaneous papillomatous cysts.

Various concentrations of the virus and antiserum are used to ascertain the dilution necessary for maximum precipitation in the radial immunodiffusion test.

MAJOR FINDINGS: Preliminary findings suggest that the radial immunodiffusion test, or a modification thereof, will produce a method to quantitate the virus and antiserum.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the intention of the investigator to use a modification of the radial immunodiffusion test in studies related to environmental pollutants to determine the effect of pollutants on the immunologic response in animals. The radial immunodiffusion test is currently being refined.

PROPOSED COURSE: To continue to explore variations of the RID test to increase sensitivity.

Serial No.: NIEHS-AST-018
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effect of Polychlorinated Biphenyls on the Immunologic Response of Rabbits"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Joe Zinkl, D.V.M., Ph.D., Animal Science and Technology Branch, and Lawrence Fishbein, Ph.D., Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	0.9
Professional:	0.2
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: To ascertain the pathologic, immunologic and enzymatic effect different aroclors of PCB have in the rabbit. Tissues will be assayed for the PCB aroclors when the experiment is terminated. An attempt will be made to correlate aroclor distribution with enzymatic, immunologic and pathologic findings.

METHODS EMPLOYED: Three hundred milligrams of PCB aroclors 1221, 1242 or 1254 are administered orally once a week to rabbits for 13 consecutive weeks. Commencing at 10 weeks the rabbits will be inoculated three times with formalinized Pseudorabies virus at 7-day intervals. Serum will be collected prior to the first inoculation, 7 and 24 days later. Serum neutralization tests utilizing the plaque reduction method will determine the titer of the antiserums from the pseudorabies inoculation. A 50 percent reduction in plaques will serve as the end point for the titer. Tissues will be submitted for histologic and electron microscopic examination.

MAJOR FINDINGS: The study is in progress and no findings can be reported at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: To characterize the effects and distribution of certain purified aroclors of PCB's

in rabbits. This experiment could set guide lines for minimal effect studies of PCB's.

PROPOSED COURSE: To complete the experiment as planned and evaluate the data for publication.

Serial No.: NIEHS-AST-019
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Hematologic and Clinical Chemical Effect of Polychlorinated Biphenyls in the Rabbit"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Loren D. Koller, D.V.M., Ph.D., Animal Science and Technology Branch, and Lawrence Fishbein, Ph.D., Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To determine the hematologic, clinical chemical and porphyrinogenic effects of different aroclors of PCB's in the rabbit.

METHODS EMPLOYED: PCB aroclor 1221, 1242 and 1254 are given orally once a week at a dose of 300 mg per rabbit. During the dosage period, multiple clinical chemical and hematologic parameters are determined. After 14 weeks, the animals are killed; liver α-aminolevulinic acid synthetase (ALA-S) activity will be determined.

MAJOR FINDINGS: Preliminary results indicate circulating SGPT and SGOT levels are increased in the rabbits receiving aroclors 1242 and 1254, but not aroclor 1221. Serum cholesterol is also increased in the rabbits receiving aroclor 1254.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An apparent difference in toxicities of PCB is suggested. This finding would suggest that relatively non-toxic PCB's could replace the more toxic compounds used by industry.

The differences in apparent toxicity might be related to differences in absorption, distribution, metabolism or excretion of these compounds.

PROPOSED COURSE: To set up guide lines for long-term, low-level studies in order to most effectively evaluate the chronic effects.

Serial No.: NIEHS-AST-020
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effect of Acute Starvation After Long-Term, Low-Level Feeding of a Polychlorinated Biphenyl in the Virginia Opossum (Didelphis virginiana)"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: Lawrence Fishbein, Ph.D.
Loren D. Koller, D.V.M., Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To study the effects of mobilization of body fat starvation after long-term feeding of PCB.

METHODS EMPLOYED: Captive born opossums will be given PCB, aroclor 1254, at doses of 0.1 and 1.0 mg/day in the diet. At monthly intervals, hematology and clinical chemistry will be determined in order to monitor the continued effect of these doses. After several months, some of the animals will be deprived of food and the hematology and clinical chemical parameters determined. The animals will be killed along with control and unstarved PCB-fed animals for histopathological and chemical evaluation.

MAJOR FINDINGS: It is too early to report any findings.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Caloric deprivation occurs in man at various intervals. In addition, anorexia commonly occurs in people with severe illnesses. If these people have PCB's stored in their fat tissue, it might be mobilized along with the fat with disastrous consequences. (The opossum is chosen for this experiment because it becomes obese when given food ad libitum when confined.)

PROPOSED COURSE: This project will be continued in an effort to determine the

PROPOSED COURSE: Research will continue in an effort to determine the effect of different aroclors of PCB in mice inoculated with dilutions of MLV.

Serial No.: NIEHS-AST-022
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Evaluation of Teratogenic Effects of Hycanthone Methanesulfonate and Selected Analogs in the Mouse"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.45
Professional:	0.25
Other:	0.20

PROJECT DESCRIPTION

OBJECTIVES: To determine if hycanthone methanesulfonate, a newly discovered mutagen, induces congenital malformations in mice.

METHODS EMPLOYED: Date bred pregnant mice received an intramuscular dose of either 10, 35 or 50 mg/kg on gestation day 7. An additional group received an IM dose of 50 mg/kg on days 7, 8 and 9. Appropriate controls received an IM dose of sterile water for injection. Animals were killed on gestation day 17; pups were removed by cesarean section, approximately two pups for each litter were processed for examination of skeletal malformations. The remainder were examined for visceral defects by gross serial section. Standard teratological procedures were employed in compiling and evaluating the data.

MAJOR FINDINGS: Hycanthone methanesulfonate, 50 mg/kg, administered on days 7, 8 and 9 caused almost total fetal resorption. Approximately 50% fetal mortality was observed at the single 50 mg/kg dose. Fetal body weight was decreased at the 35 and 50 mg/kg dose level. At the 35 and 50 mg/kg dose, malformations occurred in more than three-fourths of the litters with the percentage of abnormal fetuses in a litter increasing with dose. Three visceral malformations, exencephaly, hydrocephaly and microphthalmia, were consistently seen. The number of litters involved and the percentage of fetuses within a litter with exencephaly were greatest at the high dose.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Finding

hycanthone methanesulfonate, a drug used in the treatment of schistosomiasis, to be a potent teratogen in mice is the first indication of the drug's potential for the production of congenital anomalies. Its teratogenic properties in other mammalian species should be investigated so that consideration may be given towards evaluating the efficacy of the drug's administration to pregnant women. These findings suggest that there appears to be a positive correlation between mutagenicity and teratogenicity of a chemical.

PROPOSED COURSE: Analogs of hycanthone, retaining schistosomicidal activity but with no apparent mutagenic effects, are to be tested for their teratogenic potential.

PUBLICATIONS

Moore, John A.: Teratogenicity of Hycanthone in Mice. Nature. (In Press)

Serial No.: NIEHS-AST-023
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D.
Joseph Zinkl, D.V.M., Ph.D.
Joseph Haseman, Ph.D., Biometry Branch

COOPERATING UNITS: None

MAN YEARS

Total:	1.7
Professional:	0.6
Other:	1.1

PROJECT DESCRIPTION

OBJECTIVES: Continuous treatment of pregnant mice with TCDD results in production of cleft palate and hydronephrosis in their offspring. This study is to specifically focus on the following:

1. Characterize the stages of development during which the rodent fetus is susceptible to TCDD.
2. Characterize the pathogenesis of renal malformations due to TCDD treatment.

METHODS EMPLOYED: Using timed pregnant C57Bl/6 mice, varying doses of TCDD are administered per os at specific periods of gestation. Treated females are killed at various intervals; the fetuses are harvested and subsequently examined. In postnatal studies, reciprocal cross foster manipulations are used to determine renal effect due to *in utero* exposure, postnatal exposure or combined exposure.

MAJOR FINDINGS: The incidence of cleft palate and kidney anomalies is not diminished when treatment of pregnant mice is restricted to gestation days 10-13. There is a marked decrease in cleft palate incidence when dose is decreased from 3 µg/kg to 1 µg/kg. Cleft palate is not produced at the lower dose when administered on day 10. There is a decrease in incidence of kidney anomalies as dose and frequency of administration is reduced. TCDD administered at 1 µg/kg on day 10 still results in the detection of kidney aberrations in 33%

of fetuses. Pilot postnatal experiments show that neonates exposed in utero or postnatally develop polycystic kidneys. In utero and postnatal exposure produces an additive effect.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The etiology of the vast majority of renal malformation remains obscure. Determining the mechanism by which TCDD produces this effect may help define factors which lead to kidney malformation. The exquisite susceptibility of the developing C57Bl/6 mouse kidney to TCDD affords a reproducible system to study this disease.

PROPOSED COURSE: Project to continue.

Serial No.: NIEHS-AST-024
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Incidence of Hydronephrosis in C57Bl/6 Mice"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the incidence of spontaneous kidney anomalies in C57Bl/6 mice of different age groups.

METHODS EMPLOYED: A total of 3074 mice of different age groups were euthanatized by decapitation. Kidneys, ureters and bladder were examined grossly for any abnormality.

MAJOR FINDINGS: Hydronephrosis and cystic kidneys were observed in 1.7 and 1.2% of the mice examined at 1 and 2 weeks of age respectively (Table 1)*. The incidence was lower in 0-week (0.4%) and 13- to 24-week-old (0.2%) mice. No kidney lesions were observed in 3- and 4-week old mice. However, the number of animals examined in these age groups was very small. The right kidney was more often involved than the left one. Only one kidney was affected in all instances. *(See Table 1 on page 2Q3.)

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: C57Bl/6 mice are routinely used to study the teratogenic effect of certain compounds at this Institute. Kidney anomalies in fetal and neonatal mice are one of the parameters employed to gauge the teratogenicity of a compound. The spontaneous incidence of kidney abnomalites must be known so that it may serve as a basis for comparison.

PROPOSED COURSE: About 1000 3- and 4-week-old mice will be examined for kidney anomalies to complete this investigation.

Table 1. Incidence of Hydronephrosis in C57B1/6 Mice

Ages (weeks)	No. of Mice Examined	No. of Mice Affected	Percentage of Mice Affected
0	683	3	0.439
1	347	6	1.724
2	737	9	1.221
3	180	0	0.000
4	9	0	0.000
13-24	1118	2	0.178
TOTAL	3074	20	0.651

Serial No.: NIEHS-AST-025
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Comparative Effects of Selected Detergent Formulations on the Rabbit Eye"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: John A. Moore, D.V.M.

COOPERATING UNITS: Industrial Biotest Laboratories, Inc.
Northbrook, Illinois

MAN YEARS

Total:	0.25
Professional:	0.15
Other:	0.10

PROJECT DESCRIPTION

OBJECTIVES: To determine the extent of eye damage following instillation of phosphate, carbonate or metasilicate containing detergents into the rabbit eye.

METHODS EMPLOYED: The FDA Standard Eye Test was modified by extending the observation period to 14 days. The detergents, in dry form (0.1 ml), were inserted between the lower lid and cornea; the eye was gently held shut for one second and not washed. Control and experimental eyes were graded for extent of damage (Draize) and photographed. In selected instances, both eyes (experiment and opposite control) were removed and examined for histopathologic changes. Nine commercial products were tested.

MAJOR FINDINGS: There appeared to be, with minor variations, a positive correlation between the type and extent of eye damage observed with the composition of the products active ingredient. All products produced significant eye irritation. Major significance, however, was placed on the potential for producing residual corneal damage because this type of lesion would impair vision.

The phosphate containing detergents caused a transient superficial corneal opacity which existed a maximum of 42 hours. All eyes were found to be essentially normal by the end of the 14-day observation period.

Eyes treated with a carbonate containing detergent typically developed a marked

corneal opacity which persisted throughout the observation period. In some cases, the integrity of the corneal surface was compromised with the production of a desmetocele.

Metasilicate containing detergents are characterized by their marked caustic properties which produce irreversible eye damage. At the end of the 14-day observation period, either total eye destruction or complete corneal opacity was observed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study demonstrates that certain household detergents present a real hazard in their potential ability to produce permanent eye damage.

PROPOSED COURSE: Project completed.

Serial No.: NIEHS-AST-026
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Transmission and Pathogenesis of Encephalitozoon cuniculi in Laboratory Rabbits"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Dr. John Shadduck, Ohio State University

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Attempt to determine route of transmission and pathogenesis of E. cuniculi in laboratory rabbits.

METHODS EMPLOYED: 1×10^6 E. cuniculi organisms grown in rabbit choroid plexus cells will be inoculated intracerebrally in 3 rabbits as a control for infection and orally in 7 rabbits to determine if transmission will occur by the oral route. Two-month-old New Zealand White rabbits will be used.

The intracerebral, intravenous, intraperitoneal and subcutaneous routes of inoculation will also be investigated if rabbits can be infected orally. A pre-inoculation skin test will eliminate positive animals from the experiment. Skin test will be performed at 3-day intervals for two weeks and then weekly thereafter. Uninoculated rabbits will serve as controls for the skin test.

An animal from each group will be killed at 1, 3, 5, 7, 9, 11, 14, 17, 21, 24 and 28 days and 2 animals from each group at 1-1/2, 2 and 3 months post-inoculation. Various tissues will be saved for histopathology and also frozen for future fluorescent antibody tests should a test be developed. Serum will be collected and saved for future immunologic determination.

MAJOR FINDINGS: It is too early to report any findings.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An

understanding of the transmission and pathogenesis of E. cuniculi in rabbits and the development of a possible immunologic test for detection of diseased rabbits will allow infected rabbits to be identified and removed from animal colonies. The opportunity to use rabbits free of the disease would enable investigators to include the brain and kidney in evaluating experimental data.

PROPOSED COURSE: Effects of different doses of the organism on animals at various ages should be examined; a sex, species and breeding preference should also be determined.

Serial No.: NIEHS-AST-027
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Congenital Transmission of Encephalitozoon cuniculi in the Rabbit"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Dr. John Shadduck, Ohio State University

MAN YEARS

Total:	0.9
Professional:	0.6
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To determine if E. cuniculi is naturally transmitted congenitally.

METHODS EMPLOYED: Five groups of 3 adult female rabbits, which are non-reactive to Encephalitozoon (skin test), will be experimentally infected at various intervals prior to and after mating. One doe from each group will be killed one day prior to kindling and the young examined for lesions employing a histological and a fluorescent antibody technique. The remaining young will nurse either on their maternal doe or foster non-reactive does to preclude lactation transmission. A reciprocal cross foster will determine infectivity during the nursing period. All offspring will be skin tested and necropsied at intervals until 6 months of age.

MAJOR FINDINGS: It is too early in the project to report any findings.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Encephalitozoonosis has been detected in our research rabbits, producing renal and cerebral lesions. Though clinically silent, this infection must be considered an additional experimental variable; therefore, elimination of reactive animals is desirable. Efforts have begun to screen out reactive animals, but it is necessary to determine if offspring are congenitally infected as the young are prominently used in experimentation.

PROPOSED COURSE: To continue research to determine if E. cuniculi is naturally transmitted congenitally.

Serial No.: NIEHS-AST-028
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Growth of Rabbit Choroid Plexus Cells in Tissue Culture"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: John A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	0.55
Professional:	0.05
Other:	0.50

PROJECT DESCRIPTION

OBJECTIVES: To grow and reproduce rabbit choroid plexus cells in tissue culture. The cells will be used for the propagation of Encephalitozoon cuniculi.

METHODS EMPLOYED: Choroid plexi were removed from the brains of young rabbits using sterile procedures. Attempts to grow the cells using the previously documented plasma clot method gave unsatisfactory results. Different variations of the above procedure were then attempted. The optimum procedure was to trypsinize the choroid plexus, centrifuge to remove the trypsin and place the cells in a Falcon tissue culture flask with MEM media.

MAJOR FINDINGS: The most rapid and successful method of producing a monolayer of rabbit choroid plexus cells was by treating with trypsin and then adding MEM media. Monolayers resulted as early as 7 days post culture. The cultures have been passed as many as seven times. Storage at -70 C for up to three months did not injure the cells.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The rabbit choroid plexus tissue cultures will be used to grow E. cuniculi protozoan for investigative purposes (see NIEHS-AST-026). Pseudorabies virus will grow in the cells while bovine papilloma virus did not. Other viruses, especially those involving the endothelium (canine hepatitis, etc.), will be examined for growth in the choroid plexus cells.

PROPOSED COURSE: To continue producing monolayers of rabbit choroid plexus for future use in propagating E. cuniculi.

Serial No.: NIEHS-AST-029
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effect of Lead, Cadmium and Mercury on the Immunologic Response of Rabbits"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.2
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To determine the effect lead, cadmium and mercury, at a sublethal dose, would have on the immunologic response in rabbits when exposed to a viral antigen.

METHODS EMPLOYED: The metals were placed in deionized water and fed daily for 10 weeks. The concentration of the metals were: Lead Acetate - 250 mg/100 cc water, Cadmium Chloride - 30 mg/100 cc water and Mercuric Chloride - 1 mg/100 cc water. After 10 weeks, the metals were removed from the water, and the rabbits were inoculated with formalinized pseudorabies virus. They were subsequently inoculated 7 and 14 days later.

The rabbits were weighed and bled weekly. Serum proteins will be determined. A pre-inoculation and 7- and 24-day post inoculation serums will be examined for titer to pseudorabies virus utilizing the plaque reduction test. Whole blood will be assayed for the appropriate metal.

The rabbits, and an additional 10 non-immunized rabbits, were challenged after bleeding at 24 days post inoculation with a 100 percent lethal dose viable pseudorabies virus.

Tissues were collected for histopathology and frozen for possible fluorescent antibody studies.

MAJOR FINDINGS: Data from the weekly hematocrits and weights as well as histopathology indicate that lead had the most pronounced and mercury the least effect when compared to control rabbits. Preliminary studies indicate that the antibody titer from all treated rabbits will be less than those of the control rabbits.

All of the uninoculated rabbits died when challenged with pseudorabies virus while approximately 1/3 of the inoculated rabbits (including controls) died. There did not appear to be a difference in protection of the animal to pseudorabies virus when exposed to the lead, cadmium or mercury.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A decrease in antibody titer in the heavy metal treated animals would indicate a suppression of the immunologic system and perhaps render the animal more susceptible to other infections.

PROPOSED COURSE: If there is a decrease in the immunologic response of animals with heavy metals, a study may be originated using smaller concentrations of the metals until "no response" effect is noticed.

Serial No.: NIEHS-AST-030
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Environmental Chemicals on Experimental Viral Infections in Mice"

PREVIOUS SERIAL NUMBER: NIEHS-AST-001-2

PRINCIPAL INVESTIGATOR: Joseph H. Gainer, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.97
Professional:	0.97
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To test for interactions in mice between the salts of metals and viral infections.

METHODS EMPLOYED: Four-week-old CD-1 male mice were treated continuously with metal salts in their drinking water. At 6 weeks of age they were inoculated with an LD_{10-20} of the encephalomyocarditis virus (EMCV) or with the Rauscher leukemogenic virus (RLV).

When compounds were found that altered the viral disease, studies were performed to delineate mechanisms--such as examination for viral titers, viral adsorption rates in tissue cultures and determination of interferon titers. Assays of tissues for concentrations of metals have been performed in certain instances.

MAJOR FINDINGS: Treatment of mice with cadmium sulfate, lead acetate, mercuric chloride or nickel sulfate has increased EMCV mortality. The enhanced mortality by cadmium, lead and nickel was directly proportional to the concentration of the compound administered. Enhanced mortality with mercuric chloride was somewhat unique in that variable concentrations of the compound did not appear to affect viral mortality, but viral inoculum appeared to be more critical; in fact, a threshold of viral inoculum was needed before the mercuric chloride had an effect. Of these 4 metal salts tested, lead acetate was the most potent enhancer of EMCV mortality.

In an indirect fashion, the treatment with lead acetate was shown to enhance the EMCV mortality through depressed interferon function. Leaded mice were not protected from EMCV mortality by poly I:poly C; whereas, cadmium, mercury and nickel did not influence this mechanism.

Several metals were tested for their effects on RLV infections as determined by the development of splenomegalies and viral titrations. Salts of arsenic, cadmium, cobalt, copper, iron, lead, mercury and nickel were used. Spleens of control mice were essentially non-splenomegalic indicating that these crossbred CD-1 mice were refractory to infection by this virus. Spleens of certain groups were significantly larger than controls as follows: lead ($p<.01$), mercury ($p<.08$), and cobalt ($p<.02$). Spleens of the organic arsenical 4 hydroxy-3-nitrobenzenearsonic acid were slightly smaller than controls ($p<.09$). Leaded-viral spleens weighed 4-1/2 to 7-1/2 times the control spleens; cobalted-viral spleens weighed 2 to 4-1/2 times more than controls. No virus was detected in the control spleens whereas 250 to 1000 plaques of virus were detected in the leaded-viral spleens.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data presented indicates that several metals (particularly lead) are of danger from the view of increasing viral-induced disease. These results indicated the need to reduce environmental contamination with metals so that man and animals are exposed to lower concentrations.

PROPOSED COURSE: To complete studies on the interactions of lead with interferon formation and action and with the poly I:poly C protective system against viral infections in mice. To study manganese poisoning and EMCV and RLV infection in mice. To undertake, if possible, a cooperative project with Dr. Glen Fairchild (EPA, Research Triangle Park) on the interaction of lead poisoning and infection with the tubercle bacillus; both lead poisoning and tuberculosis are common in the inner cities. Are there interactions between these two factors? To examine further the interactions between metal poisonings and enhanced RLV infections in mice, such as: (1) Is the apparent natural resistance by the CD-1 mouse altered by metals through reduced interferon activity? (2) What effect does cross-breeding CD-1 mice to Balb/C (RLV susceptibles) have on the response of the offspring to metals and RLV infections? (3) How do the Balb/C mice respond to metals and RLV infection?

PUBLICATIONS

Gainer, J. H., Long, J. J., Jr., Capps, W. I. and Hill, P. R.: Inactivation of the Pseudorabies Virus by Dithiothreitol. Virology. 45: 91-100, 1971.

CELL BIOLOGY BRANCH

CELL BIOLOGY BRANCH
Summary Statement

Mycotoxicology

The aims of the mycotoxicology program are to identify potentially hazardous fungi, to isolate and identify the more important toxins from toxigenic species, to develop methodology for quantitative analysis of the toxins and to determine their toxicological properties in mammalian cells and in experimental animals.

Some of the work was deferred this year due to Dr. Pero's absence. However, the efforts toward isolation and identification of new toxins and development of analytical methodology were continued. A new toxin from Alternaria tenuis, strain M_1, was isolated and its structure determined except for alternative placement of a hydroxyl and methoxyl group. The compound is an analog of alternariol monomethyl ether. It is a member of a family of α-pyrones produced by this organism, apparently through the mechanism of polyketide condensation.

Two new moderately toxic compounds were isolated from another Alternaria spp.; e.g., A. mali, strain A_3. Both of these have been paritally characterized. They have the empirical formulae C_{20} H_{16} D_6 and C_{20} H_{14} O_6. The formulae and various physical chemical characteristics suggest that they are closely related compounds differing only with respect to two protons. Their characteristics closely parallel those of xanthones and anthones. Determination of precise structures, however, requires further work and probably X-ray analysis in order to establish the sites of hydroxyl groups, double bonds and the fourth ring. The compounds do not correspond precisely in physical characteristics to any xanthones or anthones available as models.

The screening program for new toxigenic fungi yielded a number of toxic isolates from commercial macaroni, rice, corn meal and wheat flour. The toxins from these will be studied as time permits. It may be significant that one of the isolates from macaroni appears to produce aflatoxins B_1 and B_2 and was tentatively identified as Aspergillus flavus.

Efforts were continued to develop better methods for isolation and quantitative assay of alternariol, alternariol monomethyl ether, altenuene, patulin and penicillic acid. Satisfactory methods for isolation and crystallization of all of these compounds were developed. In addition, a gas chromatographic procedure was developed for quantitative assay of the five toxins simultaneously in crude fungal extracts. The limits of detection for the different compounds are 0.025 to 0.25 ug.

Attempts to utilize gas chromatography as a basis for analysis of the new toxins from Alternaria mali were unsuccessful. However, because both compounds fluoresce strongly under UV, a quantitative thin layer chromatography procedure similar to that in use for aflatoxins should be quite feasible and satisfactory.

Studies were continued to determine the influence of some commercially important fungicidal compounds on the morphological characteristics and toxin producing capabilities of Alternaria mali, strain A_3. Six morphological variants were found upon treatment with zineb, 5 with maneb, 4 with nabam, 3 with ferbam, 2 with thiram and 2 with captan. Because the variants were derived from a single nuclear type and no variants were observed in the controls, they are considered to be mutants.

Toxin production by the variants varied widely, from one isolate which was as toxic to mice as the wild type (LD_{50} 300 mg/kg), to one which was not lethal at 2400 mg/kg. It was found also that vapors from deposits (presumably decomposition products) of all the fungicides greatly reduced toxin production.

Because of the variability within species of the genus Alternaria and the great difficulty encountered in taxonomic work with this organism, studies were made on the feasibility of using spore wall morphology at the ultrastructural level as a reliable means of distinguishing strains, species, etc. With seven different isolates, it was found that the surface structure of the spore walls ranged from slightly roughened to highly verrucose. However, variation of the spores from a single isolate was greater than between isolates so that differentiation of isolates by this method is not feasible.

Effects of Mercurials on Protein Synthesis

Several reports in the recent literature have indicated that inhibition of protein synthesis may be a primary factor in the effects of mercurials on the central nervous system. Work reported last year from our laboratories indicated that sensitivity of oxidative phosphorylation to mercurials is as great or greater than that of protein synthesis and, therefore, may be involved in, or account completely for, observed effects on protein synthesis and the CNS. The experiments this year show that effects of different mercurials on protein synthesis in different organs is a simple function of mercury levels, which varies from organ to organ because of differential distribution. It was found, for example, that following administration of methylmercury at dosages resulting in liver or brain levels below 0.4 ug/mg protein, protein synthetic activity was increased. At the same time, in the kidney where mercury accumulated, protein synthesis was inhibited.

In cell free systems, where distribution and stability differences are largely negated, we found no appreciable difference in the amount of the different mercurials tested necessary to inhibit protein synthesis. Methylmercuric hydroxide, phenylmercuric acetate and mercuric chloride were equally inhibitory at 10 to 15 μ moles. Preliminary experiments comparing the sensitivity of protein synthesis in vitro by ribosomes and oxidative phosphorylation by mitochondria indicate that the latter is somewhat more

sensitive. This suggests that inhibition of ATP synthesis required for protein synthesis may be the primary factor in the observed inhibition of protein synthesis in the CNS at threshhold toxic levels of mercury. Further work is required to clarify this point.

Serial No.: NIEHS-CB-015
1. Cell Biology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effect of Aryl and Alkyl Mecurials on Mammalian Cell-Free System Protein Synthesis"

PREVIOUS SERIAL NUMBER: Same

PRINCIPLE INVESTIGATORS: P. E. Brubaker, Ph.D., M. E. Long, L. T. Alexander

OTHER INVESTIGATORS: R. G. Owens, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.50
Professional:	0.25
Other:	0.25

PROJECT DESCRIPTION

OBJECTIVES: Several reports in the recent literature have provided biochemical and morphological evidence that protein synthesis is a major molecular disturbance in the central nervous system of methylmercury poisoned experimental animals. Investigations in this laboratory have shown that effects on protein synthesis in asymptomatic methylmercury poisoned rats varied with the distribution of mercury among various organs. Induction of protein synthesis activity was observed in the liver and brain at tissue mercury levels below 0.4 ug Hg/mg protein, while inhibitory effects were observed in the kidneys where mercury levels were largely in access of this level. The difference in toxicity between aryl and alkylmercurial is attributed to the in vivo stability of the C-Hg bond in the latter group of compounds. Should the cleavage of the phenyl-mercury bond be blocked there may be no difference in target organs and overall toxicity of the two groups of compounds. This reports attempts to distinguish differences in quantity and molecular effects in vitro.

METHODS EMPLOYED: There was no apparent difference in the amount of mercurial compounds required to inhibit protein synthesis in vitro. Approximately 10-15 μ moles of either phenylmercuric acetate, methylmercury hydroxide or mercuric chloride is required to inhibit the incorporation of amino acids into protein. This effect was also observed with mammalian ribosomes programed with a synthetic messenger RNA template; poly uridylic acid (poly U)

codes specifically for phenylalanine. These compounds apparently do not disturb the fidelity of the translation readout process since "poly U" programed ribosomes did not incorporate valine or leucine.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because there was no apparent difference of *in vitro* protein synthesis to the various alkylmercurials, the difference in toxicology of these compounds apparently rests upon differences in metabolic distribution, in the ligands of these compounds formed *in vivo* and the stability of the ligand-mercurial bonds. This does not resolve the problems regarding selective inhibition of protein synthesis over that of mitochondrial function as reported in the literature by the Japanese investigators. This system of *in vitro* protein synthesis has use in screening other environmental agents for their effects on fidelity in the translation of genetic information.

PROPOSED COURSE: This research will continue in an effort to distinguish differences in quantity and molecular effects *in vitro* between aryl and alkylmercurials.

Serial No.: NIEHS-CB-032
1. Cell Biology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Fungicides on Fungi and Their Toxicogenic Properties"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Miriam K. Slifkin, Ph.D.

OTHER INVESTIGATORS: Anna Ottolenghi, M.D., Hayes Brown

MAN YEARS

Total:	1.1
Professional:	1.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Fungicides are used extensively to eliminate or reduce the growth of fungi in the production and storage of food products; however, little is known of the effect these chemicals have on the fungi they do not eliminate. There are 4 ways in which the fungicides may affect the surviving organisms: (1) mutation (2) heterokaryosis, (3) various recombination mechanisms with or without a sexual stage and (4) systems of adaptation. The objective of this study is to demonstrate such changes and to determine the extent to which toxin production is enhanced or diminished in Alternaria mali (A3), an organism known to have a toxic metabolite(s).

METHODS EMPLOYED: The fungicides used were maneb, zineb, nabam, captan and folpet. A monokaryotic isolate of A3 was used throughout these studies.

To obtain variants, an aqueous suspension of each of the fungicides was spread evenly over the surface of large petri dishes containing Sabouraud's (SAB) and cornmeal agar (CMA) media. After the water soaked into the agar, approximately 500 conidia per plate were introduced. A total of 20,000 spores per fungicide were exposed in this manner. Any well established colonies were transferred to CMA, SAB, potato dextrose, malt extract and Czapek's agars. Colonies were considered mutants if, after 5 generations on each of the 5 different media, conidial production and cultural characteristics remained constant and were different from the original A3.

The effect of fungicide decomposition products was determined by counting the number of spores produced per plate in a haemocytometer; viability was determined by degree of conidial germination.

To determine if adaptation would occur, conidia were spread over the surface of large petri dishes of CMA that had a 1 cm. diameter filter paper covered with fungicide in the center. The mycelia growing nearest the fungicide were cut out and spread on a fresh plate containing the chemical. This was repeated 10 times.

All test variants were grown in rice-czapek's medium for two weeks. Crude extracts were made with 2% methanol in chloroform. In the case of A3 exposed to fungicide vapors, extracts were made of the A3 growing on SAB in presence of the fungicides with 2% methanol in chloroform. Special care was taken to insure that no fungicide contaminated the extract. The extracts were tested on 5 different bacteria and on female swiss albino mice. Because the nature of the toxin is not known, there is a possibility that any test using an extract could give a false evaluation of potency. To avoid this problem tests with bacteria were carried out with living organisms as well as with the crude extracts.

The dried extracts were tested by thin layer and gas chromotography to determine whether the mycotoxins altenuene, alternariol, and alternariol monomethyl ether were present.

MAJOR FINDINGS: Twenty-two variants were obtained when 120,000 homokaryotic conidia of Alternaria mali were exposed to 6 different fungicides. Six variants were induced by zineb, 5 by maneb, 4 by nabam, 3 by ferbam, 2 by thiram and 2 by captan. The variants were morphologically distinguishable on 5 different media on the basis of colony size, texture and color. Conidial production could also be used to differentiate the variants, but conidial ornamentation was not useful. After 10 generations in the presence of the fungicide, there was no evidence of adaptation. In the presence of all fungicide decomposition products germination of condia was greatly reduced, maneb was most inhibitory and conidial production especially in the presence of maneb and nabam was greatly enhanced. Because the variants were derived from a single nuclear type and no variants were observed in the controls, these isolates appear to be the result of mutation.

The toxin production of fungicide induced variants of Alternaria mali, when tested with mice and bacteria, varied extensively. The wild type (A3) and most variants inhibited the growth of Staphylococcus aureus, Sarcina lutea, Bacillus mycoides and B. subtilis, but did not inhibit B. megaterium. Thiram and captan-induced mutants displayed the least toxicity to bacteria. The inhibition of bacterial growth by both the living organisms and a crude methanol chloroform extract correlated well, indicating that essentially all toxic components of the cultures were extracted.

The LD_{50} of the wild type (A3) extract in female swiss albino mice was 300 mg/kg. The variants displayed varying degrees of toxicity from as toxic (A39) as A3 to not toxic enough to cause death at 2400 mg/kg (A32, A309,

A310, A311). A3 exposed to the decomposition products of maneb, zineb, ferbam, nabam, thiram and captan had dramatic toxin reduction. Mice injected i.p. with the crude extracts had no overt symptoms except for minor vacuolization of the livers.

Thin layer and gas chromatography revealed that altenuene, alternariol and alternariol monomethyl ether were not present in any of the extracts.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The more a pesticide is used, the greater the probability that some organisms survive and, hence, the greater the chance for selection of variants. Because of this selection process, there is a changing spectrum of fungi with unknown potential for toxin production. It is important, therefore, to understand the frequency of changes and the pressures that bring them about in order to evaluate fungicides as potential environmental hazards.

PROPOSED COURSE: This work is being terminated.

PUBLICATIONS

Slifkin, M. K.: Apparent induction of mutants and enhancement of conidial formation by fungicides in Alternaria mali. Mycopath. and Mycol. Appl. (In Press).

Slifkin, M. K., Ottolenghi, A. and Brown, H.: Toxin production of Alternaria mali exposed to fungicides. Mycopath. and Mycol. Appl. (In Press).

Serial No.: NIEHS-CB-034
1. Cell Biology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Toxicity, Purification and Identification of Toxins Produced by Toxigenic Fungi"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: Ronald W. Pero, Ph.D. and Robert G. Owens, Ph.D.

OTHER INVESTIGATORS: Judson W. Spalding, Ph.D.

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	2.0
Professional:	1.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: During the past decade it has become increasingly clear that many fungi associated with food crops and processed foods produce toxic compounds under conditions favorable for their growth. Some genera, such as Aspergillus, Penicillium and Fusarium, because of their ubiquity and the high frequency of toxigenicity among species and strains, have been studied extensively. Others have received relatively little attention although evidence has been accumulated over several years that some of the lesser-known genera may also produce dangerous compounds. The objective of this study is to assess the potential hazard of some of these lesser-known genera and to identify the toxins they produce. Particular attention has been given to the genus Alternaria.

METHODS EMPLOYED: Toxigenic cultures were grown in large quantities either on rice fortified with Czapek-Dox broth or in other suitable culture media by standard mycological culture techniques.

Extraction of toxins and other compounds from culture media and mycelia was done with a veriety of solvents as deemed appropriate from our own experiences or those of other natural products chemists. One of the more useful solvents for initial extraction of the compounds reported here was 70% acetone in water.

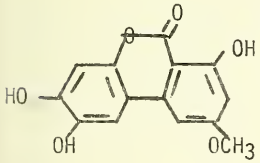
Generally, a secondary solvent fractionation was made with tetrabydrofuran (THF) to obtain subfractionations that facilitated purification.

Separation of compounds and subsequent purification of the toxins were accomplished by column and thin layer chromatography on silica gel using various mixtures of bezene and THF. Toxicity of fractions was monitored by antibacterial assay (disc method) with Bacillus mycoides, Staphyloccus aureus, Sarcina lutea or Bacillus megaterium as indicator organisms.

Identification of purified compounds involved use of IR, NMR, MS and UV spectrometry and spectrophotometry and derivatization as necessary, and will, in some cases in the future, require X-ray analysis as pointed out below.

Mammalian toxicity of crude extracts and purified toxins was determined in random bred CD-mice, hybrid DBA mice and in cultured lymphoma cells.

MAJOR FINDINGS: A new compound, altenuene, was identified last year as a toxin from a culture of Alternaria tenuis, strain M_1. This year a second new compound was isolated. The compound has slower mobility on silica gel columns and plates than altenuene in the solvent systems employed. It is a yellow amorphous powder melting at 255°C. The mass spectrum indicates the parent molecular ion [M+] of 274, consistent with the empirical formula $C_{14}H_{10}O_6$. The UV spectrum and blue fluorescence under UV radiation are very similar to those of alternariol and alternariol monomethyl ether. However, it differs from both in that it lacks the methyl group on the A ring. The IR spectrum indicates the presence of all other functional groups. NMR and molecular weight indicate three hydroxyl and one methoxyl group, so it appears to be closely related to alternariol monomethyl ether. NMR further indicates 2 para coupled and 2 meta coupled protons. Thus, three structures are possible based on these observations.



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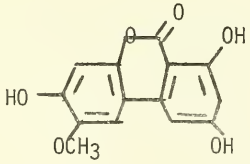

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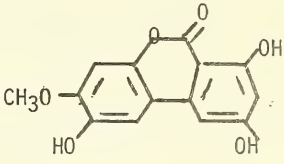


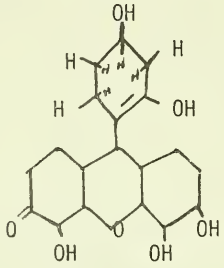
III

Structure I has been ruled out tentatively on the basis of the fact that no reaction occurred with ammonuim molybdate - a reaction characteristic of ortho dihydric phenols. Thus, the structure appears to be either II or III. Efforts are being made now to biosynthesize additional material for p - bromobenzoate derivatization and X-ray analysis for unequivocal location of the methoxyl and adjacent hydroxyl group.

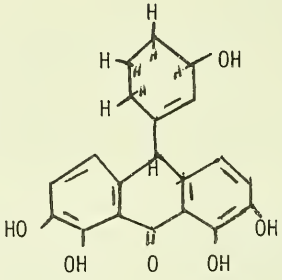
Two new compounds were isolated from Alternaria mali, strain A_3, originally obtained from apple and shown to be toxic to mice. The compounds were obtained in mixture by column chromatography on silica gel G, using THF-benezene mixture with increasing proportions of THF. The mixture was subsequently resolved into two yellow compounds by re-chromatography on silica gel G, using 30% ethylacetate in benzene. The toxins were recrystallized from THF-hexane. One compound, designated A-3-2-B, yielded orange crystals that melted with decomposition at about 190°C. This compound (B) fluoresced dark brown or black under UV radiation. The second compound (Y) decomposed at about 210°C. It fluoresced bright yellow under UV.

Mass spectrometry indicated molecular weights of 352 and 350 for B and Y, respectively, consistent with empirical formulae $C_{20}H_{16}O_6$ and $C_{20}H_{14}O_6$. Apparently Y is the dehydro form of B.

NMR spectrometry of Y indicated two sets of overlapped AB aromatic protons (ortho coupled) and 7 or 8 methylene protons (exact number obscured by overlap in the OH region; however, the MW is consistent with only 7). The IR pattern indicated limited methylene function, eliminating the likelihood of a 3-carbon aliphatic chain to account for the methylene protons. Thus, the data are consistent with a condensed ring system. MW and fluorescence characteristics resemble those of xanthones and anthones with the following general structures:



IV



V

Structure IV is in accord with the interpretation of the NMR and 7 methylene protons. Structure V, however, requires a vinyl proton which is not indicated by the NMR. Thus, our first choice is the general structure IV, a xanthone. As can be seen, however, there are a number of possibilities for placement of hydroxyl groups, double bonds and the fourth ring. Further work based on structure IV as the postulate is in progress. The numerous other structural possibilities will also be considered as further work dictates.

Attempts to use gas chromatography as the basis of analytical method development for the two compounds were unsuccessful. Even silylated derivatives failed to chromatograph at 300°C. This indicates that because

of MW and configuration even silyl derivatives have very low vapor pressures. However, because of the brilliance of fluorescence in UV, UV analysis on thin layer plates similar to that used for aflatoxins appears to be a good approach for quantitative analysis should the compounds prove to be of sufficient interest for development of an analytical procedure.

Bioassays indicate that neither of these compounds, while somewhat active, is the primary toxin of A mali. Crude extract of A_3 is active against B mycoides at 60 ug/disc, whereas purified Y and B toxins exhibit activity only in the range of 250 ug/disc. Toxin B was lethal to mice at about 80 mg/kg i.p., whereas the crude extract exhibited activity at about 400 mg/kg. These data suggest the presence of other toxins as did previous experiments. Work is continuing toward isolation of other toxins.

Some ten toxic isolates of Alternaria associated with grains were obtained from Dr. Christensen and his colleagues at the University of Minnesota. The most toxic of these, designated isolate C-3, was lethal to mice at 100 mg crude extract/kg. The fungus was examined first for known Alternaria toxins by TLC and GLC analysis. No alternariol, alternariol monomethyl either or altenuene was found. However, after subculturing for a year, cultures are now producing substantial quantities of the above toxins. Thus, freeze-dried inoculum from the original culture is being used for large-scale cultures which will be used for isolation of toxins of this strain. This work has just been initiated.

Screening of new cultures of molds from various food products is continuing. Toxic isolates have been obtained from macaroni (21 of 32 isolates toxic), rice (5 of 9), corn meal (1 of 32), wheat flour (2 of 16) and air (1 of 18). One of the isolates, tentatively identified as Aspergillus flavus, from macaroni appears to produce aflatoxins B_1 and B_2. Further work with the toxic isolates from all sources is anticipated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Work over the past decade has clearly demonstrated the potential hazard of certain molds associated with agricultural commodities. The frequency of reports of new toxins from many genera of fungi is indicative of the paucity of information about fungi that inhabit food products and the nature of the toxins they produce. It is essential, therefore, that efforts continue toward better assessment of the potential importance of fungal byproducts in foods and their long range effects, in combination with other chemical agents, on the health of man.

PROPOSED COURSE: Efforts to isolate, purify and identify toxins from significant toxigenic fungi on hand, from our screening program and from other sources will continue. In concert with this, efforts will continue to develop fuller understanding of the toxicological aspects of both known and new mycotoxins.

Serial No.: NIEHS-CB-035
1. Cell Biology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Analytical Methodology for Mycotoxins"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: Ronald W. Pero, Ph.D. and Robert G. Owens, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To develop reliable qualitative and quantitative methods for detection and measurement of mycotoxins in naturally moldy agricultural commodities, foods and mammalian fluids and tissues.

METHODS EMPLOYED: Development and refinement of analytical methodology for a number of mycotoxins was continued. Basically, the procedures were centered around partition between solvents, thin layer chromatography and gas-liquid chromatography.

MAJOR FINDINGS: Experiments were continued to find better methods for isolation and quantitative assay of alternariol (AOH), alternariol monomethyl ether (AME), altenuene, penicillic acid (PA) and patulin. A column chromatographic method for fairly large scale isolation of AOH, AME and altenuene has been worked out so that it is now possible to obtain each compound directly from the column in sufficient purity for crystallization. Column methods for cleanup of penicillic acid and patulin were also devised. However, subsequent thin layer methods are required for purification.

Several satisfactory solvent systems were found for thin layer chromatography of AOH, AME and altenuene. These include 20% THF in benzene, 5% ethanol in benzene and 5% methanol in chloroform. All of these provide good resolution of the toxins from each other and from other compounds in crude fungal extracts.

For purification of pencillic acid and patulin in extracts of fungi grown on corn or rice, 500 μ silica gel G chromatographic plates developed with 10%

methanol in chloroform provided a satisfactory means of purification of the compounds. After separation, the zones containing the compounds are scraped from the plates and the toxins eluted with acetone.

All of the five toxins can be separated and quantitated by gas chromatography. The most satisfactory of several systems tested for silyl derivatives of AOH, AME and altenuene was 3% OV-17 with a temperature program of 100 to 250° at 8°/min. The minimal detection limit for each compound under these conditions is about 0.25 μg.

The same column and program parameters were quite satisfactory for silyl derivatives of penicillic acid and patulin. In fact, because of wide differences in retention times, all of the five compounds can be quantitated simultaneously. The lower detection limits for PA and patulin were of the order of 0.025 μg.

It was found that PA could be chromatographed without derivatization under the same conditions with a detection limit of about 1 ug. While silylation increases sensitivity to PA, use of silylated and nonsilylated forms is a useful tool for indentification and confirmation of PA in crude samples.

These procedures were applied to extracts of naturally moldy commerical tobacco samples obtained from N.C. State University. No AOH, AME, altenuene or patulin was detected in the samples analyzed. However, PA was detected in tobacco naturally infested or artificially inoculated with Aspergillus ochraceus and Penicillium cyclopium. Naturally moldy tobacco contained 10 to 20 μg PA/100g. of tobacco. Levels in artificially inoculated tobacco reached 320 to 1800 μg/100g. It was shown also that as much as 3% of PA in tobacco made into cigarettes and smoked could be recovered in the smoke condensate. This finding prompted contract support of further research at N.C. State University on the occurence of PA in tobacco and in cigarette smoke. The contract was initiated January 1, 1972 and will continue through August.

Attempts to utilize gas chromatography as a basis for analysis of new toxins from Alternaria spp. (NIEHS-CB-034) were unsuccessful. However, because the compounds are strongly fluorescent under UV, a quantitative assay procedure similar to that in use for aflatoxins should be quite feasible and satisfactory.

PROPOSED COURSE: Efforts will continue toward development and refinement of sensitive quantitative analytical procedures for new toxins as they are found and for known toxins where existing procedures are inadequate.

PUBLICATIONS

Pero, R.W., Owens, R.G., and Harvan, D.: Gas and thin-layer chromatographic methods for the mycotoxins altenuene, alternariol and alternariol monomethyl ether. Anal. Biochem. 43: 80-88, 1971.

Lucas, G.B., Pero, R.W., Snow, J.P., and Harvan, D.: Analysis of tobacco for the Alternaria toxins alternariol and alternariol monomethyl ether. Agric. and Food Chem. 19: 1274-1275, 1971.

Pero, R.W., Harvan, D., Owens, R.G., and Snow, J.P.: A gas chromatographic method for the mycotoxin penicillic acid. Jour. Chromatography. 65: 501-506, 1972.

Serial No.: NIEHS-CB-038
1. Cell Biology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Studies in Molecular Cytogenetics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Ronald W. Pero, Ph.D.

OTHER INVESTIGATORS: Professor A. Lima-de-Faria

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: This work, carried out by Dr. Pero at the Institute of Molecular Cytogenetics, University of Lund, Lund, Sweden, is concerned primarily with: (1) elucidation of gene amplification in the cricket, (2) studies of the stimulation of ribosomal cistrons in human lymphocytes by phytohemaglutinin and (3) isolation of ribosomal DNA from human lymphocytes.

METHODS EMPLOYED: The principal methodology involved cesium chloride gradient and hydroxyapatite fractionation of DNA and RNA and DNA-RNA hybridization techniques. Studies were conducted on oocytes of Acheta demesticus and on human lymphocytes.

MAJOR FINDINGS: Oocytes of the cricket, Acheta domesticus, contain a nuclear DNA body not found in somatic cells. This body is postulated to be responsible for the rapid synthesis of protein. Saturation hybridization of DNA with homologous RNA from 28 and 18S ribosomes indicates that there is more DNA in oocyte ribosomes than in somatic cell ribosomes, which is consistent with the higher rate of RNA template and protein synthesis in the oocytes.

It has been demonstrated that phytohemagglutinin (PHA) will induce peripheral blood lymphocytes to divide. It was postulated that induction of mitosis in normally nonmitotic cells is due to gene amplification. However, DNA-RNA hybridization experiments, involving DNA saturation with 28S and 18S particle RNA, indicated a decrease in ribosomal DNA (genes) at stimulatory levels of PHA.

Thus, mitosis apparently is initiated by some factor other than gene amplication as originally suspected.

Efforts were made to isolate and at least partially characterize the ribosomal DNA from human lymphocytes. Normally, rDNA from lymphocytes does not appear as a conspicuous satellite upon cesium chloride density centrifugation analysis. However, hybridization of fractionated DNA with rRNA and recentrifugation yields a hybrid peak on the heavy (high cytosine and guanine) side of the bulk DNA band. Material in the hybrid peak was further fractionated on hydroxyapatite columns. It was found that fast- and intermediate-replicating DNA comprised 90% of the DNA of this fraction. The remaining 10% was made up of slowly replicating material that appeared to be unique. Hybridization of the different fractions with rRNA indicated similar saturation values for all fractions. This suggests that the rDNA is very heterogeneous and may be associated with several different classes of repetitive DNA in the usual fractionation procedures.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Nuclear and rDNA are, of course, fundamental to gene fidelity and the processes of cell growth and growth regulation. Interaction of many noxious environmental agents with these vital components is well established from the considerable work already done in mutagenesis, protein synthesis and other cellular processes. Thus, it is clear that a full understanding of the immediate and long range effects of environmental toxicants on cell function and disease processes requires a basic understanding of normal DNA makeup and replication and the manner in which the genetic information is translated into cell growth and regulatory processes.

PROPOSED COURSE: Dr. Pero will complete his stay in Sweden in August. At that time a decision will need to be made regarding future work on this project.

Serial No.: NIEHS-CB-039
1. Cell Biology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Fine Structure of Alternaria"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Miriam K. Slifkin, Ph.D.

OTHER INVESTIGATORS: Hayes Brown

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Spore morphology in many fungi is used as a taxonomic characteristic to distinguish between species. Alternaria, a genus of ubiquitious plant parasites, is difficult to classify because the variations within a single species are so great that most commonly used characters for speciation are not valid. The object of this study was to compare the spore wall morphology of several Alternaria isolates on an ultra-structural level and to report on conidial wall structure.

Other studies are being made of the internal ultrastructure of Alternaria so that a greater understanding of the processes of germination, mitosis and sporulation may be obtained.

METHODS EMPLOYED: The usual procedures for carbon replicas were followed for obtaining the conidial wall structures. Conidia were prepared for scanning EM by brushing a piece of double sticky cellophane tape over cultures containing large numbers of spores. For thin section, the mature spores were pre-treated with a 1% (W/V) aqueous solution of Adolph's Tenderizer before being fixed in gluteraldehyde, post fixed in osmium, embedded in epon, sectioned and stained with uranyl acetate and lead citrate. Germinated spores were treated in the same way except that pre-treatment with tenderizer was not necessary.

MAJOR FINDINGS: The surface structure of 7 different isolates of Alternaria ranged from slightly roughened to highly verrucose. Variation within an isolate was greater than between isolates. The conidium is completely

enveloped by an electron-dense primary wall from which coarsely granulated ornamentations protrude. The cells within the spore are delimited by a secondary wall and a 3-layered septal partition. The cells are connected by simple septal pores with associated Woronin bodies and are representative of an ascomycetous septal pore apparatus.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Some isolates of Alternaria are known to be toxin producers, but it is very difficult to assign specific names to the various isolates. There was hope that the wall structure would shed some light on this problem, but it did not.

An understanding of the normal structure of an organism is necessary before an evaluation can be made of abnormalities induced by outside agents. If further studies are made on the effect of fungicides on Alternaria, it will be easy to pinpoint the organelles involved.

PROPOSED COURSE: Studies will be completed on the ultrastructure of conidial germination and somatic nuclear division.

PUBLICATIONS

Slifkin, M.K.: Conidial wall structure and morphology of Alternaria spp. J. Elisha Mitchell Sci. Soc. 87: 231-236, 1971.

MUTAGENESIS BRANCH

MUTAGENESIS BRANCH
Summary Statement

The primary thrust of NIEHS' effort in mutagenesis is focused on the evaluation and definition of risks to both human germinal and somatic tissues. The Mutagenesis Branch, organized this year and anticipated to expand to full capacity within the next several years, has addressed itself to the problem by: (1) attempting to identify specific mutagenic hazards in man's environment, (2) developing new and comprehensive mutational assay systems having a high degree of relevance to human health, (3) developing a better understanding of the molecular basis of mutation in higher organisms and (4) attempting to understand the induction processes attributable to chemical and physical challenges.

To complement and support these efforts, the Mutagenesis Branch undertook Collaborative Research studies with Dr. H. E. Sutton (University of Texas), whose activities include the development of techniques that can be applied to the problem of monitoring human populations for somatic mutations, and with Dr. H. V. Malling at the Oak Ridge National Laboratory, who directs the Environmental Mutagen Information Center which collects, stores and disseminates information on the existence and activity of mutagens in the human environment. During Fiscal Year 1972, efforts were made to promote expansion of programs in the Collaborative Research area which are intended to become an integral part of the Branch's research activities.

It is recognized that the nature of the problems in environmental mutagenesis is so broad and requires such diverse expertise in sophisticated technology as to far exceed what any single agency or institution could alone provide. It is therefore essential that program development in the Collaborative area be a continuing effort of highest priority. It is further anticipated that efforts initiated in FY 1972 will lead to the establishment of the following Collaborative Research programs for FY 1973: (1) development of additional genetic markers for human, somatic cells (University of Wisconsin); (2) refinement and evaluation of systems capable of detecting air-borne chemical mutagens (Brookhaven Laboratories); (3) establishment of a laboratory with the capability of deducing the molecular nature of mutants induced by chemical or physical challenge; (4) investigation and characterization of virus-induced mutagenesis with special emphasis on those viruses known to contaminate pharmaceutical biologicals such as vaccines.

Within the past year, staff members of the Mutagenesis Branch have participated in varied activities including testimony before a congressional hearing (Senate Hearing before the Subcommittee on Executive Reorganization and Government Research) and presentations before the NIH Scientific Directorate and National Advisory Council, NIEHS, on the progress of research in the field of environmental mutagenesis. To help foster interest and understanding of current

problems, staff members of the Mutagenesis Branch have lectured to numerous university audiences; provided a 5 week course on "Chemical Mutagenesis" under the auspices of the Organization of American States; co-authored a book entitled "Chemical Mutagens"; organized a conference on "Detection of Somatic Mutations in Humans"; reported before the Environmental Mutagen Society; served as councilor and on the program committee of the society; chaired an open session at the 2nd Annual Meeting of the Environmental Mutagen Society; co-organized, under the aegis of the Fogarty International Center, a workshop on "Mutagenic Contaminants in the Environment"; participated in several Fogarty sponsored meetings on genetics and mutagenesis; written seven review articles describing advances in genetics and environmental research; served as program chairman of the Triangle Toxic Agent Seminar Series; reviewed numerous research and research related proposals for federal, state and private funding institutions; reviewed numerous research articles for a dozen different journals; reviewed two books on environmental mutagens for SCIENCE; served as host for a long list of distinguished scientists from this country, Scotland, England, The Netherlands, India, Germany, Brazil and Japan who presented seminars at NIEHS; and participated in joint programs with Analytical and Synthetic Chemistry, Animal Science and Technology, Pathologic Physiology and Epidemiology at NIEHS.

Considering the current limitations in the number of staff, the Mutagenesis Branch has made notable progress in its intramural research program.

A simple and highly useful assay system has been developed to study the effects of important environmental chemicals on the repair of genetic damage in human cells in tissue culture. The demonstration that N-hydroxy compounds interfere with the essential processes of the assay is of upmost importance in interpreting results derived from the employment of specific chemicals which form a ubiquitous class of carcinogens and proximate carcinogens. The correlations drawn and comparisons made between chemical and radiation damage will serve to make literature on this subject far more comprehensible.

A comprehensive study of repair events in mouse lymphoma cells is an important ongoing project which lends necessary support to our continuing effort to develop more meaningful mutational test systems employing somatic cells. Only by fully appreciating the precise nature of those events upon which mutation is predicated can a true understanding be provided as to the relevance and significance of mutational assay systems. For this reason we have undertaken highly detailed studies of the quantitative and qualitative nature of both repair and replication.

A number of methods have now been developed and refined to assess the chemical nature of genetic damage. Furthermore, it is now possible, with certain chemical mutagens, to detect genetic damage to DNA at levels of sensitivity exceeding that normally provided by use of radioactive isotopes which often are not available for these studies.

The most notable accomplishment has been the development and utilization of a new mutational assay system in mouse lymphoma cells. This work has been highly acclaimed by leading authorities in chemical and radiation mutagenesis and has lead to the identification of a drug, used extensively in Africa and

South America, as being potently mutagenic to mammalian cells. Because these observations indicate a clear need for early efforts to investigate in depth the possible teratogenic and carcinogenic potential of the drug in question, the Mutagenesis Branch has become involved in cooperative activities, coordinated by Dr. R. Tjalma (Program Planning, NIEHS), which are intended to help delineate these effects and assess the precise hazard to humans through meaningful field studies of the effects of the drug. The importance of such a comprehensive approach is in accordance with one of the primary functions of the Branch which is to develop a thorough appreciation and understanding of the implications of laboratory findings in mutagenesis to questions relating to human health.

The successful development, in mouse lymphoma cells, of two specific loci, one of which is autosomal (TK) and the other X-linked (HGPRT), should contribute much to the identification of mutagenic hazards in the environment as well as to provide an improved understanding of the types of mutations which occur in somatic cells in mammals and the mechanism by which they occur.

Considerable progress has been attained towards an understanding of G6PD variant cells in human peripheral blood. This work is being continued and other loci are also being explored pursuant to the development of cytochemical methods for assessment of somatic mutant frequencies among human populations.

The Environmental Mutagen Information Center has increased substantially its data storage files (6,000 items are now cited in the computer bank and on file) and has accumulated 2,200 documents reporting the mutagenic action of chemicals. It is expected that the 1971 compilation of literature on chemical mutagenesis will be published as a unit by November 1972.

In conclusion, the recent organization of a Mutagenesis Branch at NIEHS will ensure implementation of programs already initiated and more importantly the expansion and extension of needed programs in this critical area of environmental health science.

Serial No.: NIEHS-M-001
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Repair Replication of DNA in Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-CB-006

PRINCIPAL INVESTIGATORS: J. B. Patterson, Ph.D., W. G. Flamm, Ph.D., W. Brandt, M. D.

OTHER INVESTIGATORS: N. J. Bernheim

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The process that mediates gene repair must necessarily involve a set sequence of metabolic events. The first step involves the introduction of single-stranded breaks in DNA which facilitates the removal of the genetic lesion, such as the removal of an alkylated guanine. Secondly, the excised nucleotides are replaced by a new set of bases (but, presumably, in proper Watson-Crick register) through the function of a special repair enzyme or by the normal DNA polymerase. The last step in the process includes a resealing (esterification) of 5' and 3' ends. It is our purpose to quantitate each of the above steps so that they may be related to data on damage to genetic material and induced mutation rates. We want to know how many single-stranded breaks are produced as each lesion is removed and the number of new nucleotides inserted in response to the event. We also want to assess the fidelity of repair and to learn whether this process influences mutation rates.

METHODS EMPLOYED: The presence and extent of repair replication could be determined by the incorporation of DNA precursors into DNA were it not for the fact that two different processes are responsible for the incorporation; i.e., one involving gene-duplication and the other, repair replication. These processes, however, can be resolved because gene replication is semi-conservative while repair replication is not. In practical terms, resolution depends upon separating, on density gradients of CsCl, the molecules undergoing semi-conservative replication from those engaged in repair. This can be done by incorporation of tritiated BUdR. As a practical matter we use a

combination of BUdR and ^{3}H-labeled thymidine. Alternatively, and as described under major findings, repair replication can now be unambiguously assessed in a highly simplified assay, the basis of which depends upon inhibition of normal semi-conservative replication by hydroxyurea without quantitatively affecting repair replication.

MAJOR FINDINGS: Human cells are known to engage in repair replication to a much greater extent than cells derived from the mouse. For this reason, we turned to a HeLa cell system, which is a transformed human cell line, in order to develop and assess the value of possible analytical approaches capable of quantitating repair replication. A simplified assay for quantitating repair replication in HeLa cells was utilized which employed hydroxyurea for the purpose of selectively suppressing normal, semiconservative replication. With semiconservative replication inhibited, DNA synthesis by UV-irradiated HeLa cells (as measured by the incorporation of ^{3}H-thymidine) was shown to be a nearly exclusive product of repair replication (>80%). Thus, the highly selective action of hydroxyurea affords the opportunity of quantitating repair replication merely by assessing the rate of incorporation of ^{3}H-thymidine into DNA. Several classes of chemical compounds were studied (monofunctional alkylating agents, organochlorine insecticides, an organomercurial and N-hydroxy compounds) to ascertain whether they initiated repair replication in non-irradiated cells or inhibited the repair process once it had been initiated by the introduction of pyrimidine dimers with UV-irradiation. In those cases where initiation of repair replication was observed, attempts were made to confirm the result by isopycnic density gradient analysis in CsCl. Two monofunctional alkylating agents (methyl methanesulfonate and ethyl methanesulfonate) were confirmed to initiate repair replication while the other three classes of compounds studied proved to be negative. However, both N-hydroxy compounds tested (N-hydroxycyclohexylamine and hydroxylamine) gave falsely positive results in the simplified assay. Additional studies indicated that this artifact arose as a consequence of the N-hydroxy compounds interferring with the action of hydroxyurea; i.e., the ability of hydroxyurea to inhibit normal replication. None of the compounds tested exerted a marked inhibitory effect upon repair replication in UV-irradiated HeLa cells. Our main efforts, however, are focused on delineating repair replication events in mouse lymphoma cells for which a mutational assay system is available. The above study utilizing HeLa cells helped to provide direction and perspective toward the realization of this goal. Thus far, we have shown that monofunctional alkylating agents are capable of inducing demonstrable levels of repair replication in mouse lymphoma cells at concentrations known to mutate the TK locus. We have calculated that 2×10^{-4} mutations are induced per locus per alkylation with ethyl methanesulfonate, and we are presently working to establish the quantitative relationship between the repair of these alkylations and genetic effects.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Repair of genetic damage before the onset of irreparable genetic alteration appears to constitute the primary means by which genes are properly maintained and protected from harmful environmental influences. Employment of the above system and others in which each step of repair is individually assessed and related to the overall process should reveal which chemical and physical agents interrupt and uncouple the sequence of repair events. Such interruptions

could conceivably cause more damage to the cell's genetic material than the initial lesion. For instance, repair enzymes might produce multiple breaks in DNA and yet be prevented from resealing them by exogenous, environmental influences.

PROPOSED COURSE: Future efforts will focus on the relationship between genetic damage, repair replication and the mutagenicity of the specific chemical agents employed in the L5178Y system. Correlations will be made utilizing a series of chemicals, which should provide definite information as regards the underlying molecular mechanism responsible for mutation in mammalian somatic tissue.

PUBLICATIONS

Fishbein, L., Flamm, W. G., and Falk, L.: Chemical Mutagens: Environmental Effects on Biological Systems, New York, London, Academic Press, 1970, 360 pgs.

Flamm, W. G. and Drake, J.: The molecular basis of mutation. In Sutton, H. E. and Harris, M. I. (Eds.) Mutagenic Effects of Environmental Contaminants. Academic Press, New York, 1972, pgs. 3-17.

Brandt, W. N., Flamm, W. G. and Bernheim, N. J.: The value of hydroxyurea in assessing repair replication of DNA in HeLa cells. Chemico-Biological Interactions. (In Press).

Serial No.: NIEHS-M-002
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Interaction of Environmental Agents with DNA"

PREVIOUS SERIAL NUMBER: NIEHS-CB-010

PRINCIPAL INVESTIGATORS: W. G. Flamm, Ph.D. and J. B. Patterson, Ph.D.

OTHER INVESTIGATORS: N. J. Bernheim and L. Fishbein, Ph.D.

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	.6
Professional:	.3
Other:	.3

PROJECT DESCRIPTION

OBJECTIVES: Certain classes of environmental agents interact either physically or chemically with DNA. Such classes include alkylating agents, certain N-hydroxy derivatives, nitrous acid and nitrites, bisulfites, certain analogs of purines and pyrimidines, a variety of antibiotics and mycotoxins, heavy metal ions and various polycyclic aromatic compounds containing either oxygen, nitrogen or sulfur in the ring system. In addition, other compounds such as polycyclic hydrocarbons (dimethylbenzanthracene), heterocyclics (mitomycin C) and nitrosamines (dimethylnitrosamine) are metabolically converted to substances which attack nucleotide bases in DNA. It has been our purpose to devise a series of procedures by which the interaction of various environmental agents with DNA can be detected and the nature of the reaction products characterized.

METHODS EMPLOYED: Most mutagens that have been studied tend to react with purines to a much greater extent than they do with pyrimidines. Because the reacted purine is unstable, depurination occurs at that site, rendering the attendant phosphodiester bond alkali-labile. The rate and extent to which bond lability occurs can be accurately assessed by sedimenting single-stranded DNA (alkaline) under strictly defined conditions of salt, pH and temperature. We have also employed a nitrocellulose filter technique whereby a denatured DNA sample is filtered allowing the released purine residue with its covalently-bonded radioactive alkyl group to pass through. Comparisons are made between counts remaining on the filter and counts in the filtrate. The two methods discussed here yield grossly comparable results, however, the sedimentation method is infinitely more sensitive to very low levels of depurination.

MAJOR FINDINGS: We have determined, both in vivo and in vitro, the rate and extent to which ethyl methanesulfonate reacts with DNA as well as the rate at which it depurinates. We have also shown unequivocally that depurination provides alkali-labile sites which can be measured with extraordinary sensitivity by band sedimentation analysis in the analytical centrifuge. This method is applicable to the study of a broad class of alkylating agents which have specific affinity for the N7 atom of guanine. The mutagenicity of ethyl methanesulfonate has been quantitated in terms of induced mutation rate per alkylation per gene and has been estimated to be 2×10^{-4}.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In mammalian systems, all known mutagens appear to function by interacting with DNA and in many instances new covalent bonds are formed. By examining whether certain untested, environmental compounds and their metabolites interact with DNA and the conditions under which the reaction takes place, we derive information relative to their potential mutagenicity.

PROPOSED COURSE: Attempts will be made to utilize this highly sensitive procedure for determining whether compounds of great environmental importance have the potential for producing genetic damage. High on our list of compounds requiring study are fumigants and volatile pesticides.

PUBLICATIONS

Flamm, W. G., Birnsteil, M. L. and Walker, P. M. B.: Isopycnic centrifugation of DNA, methods and applications. In Birnie, G. D. and Fox, S. (Eds.) Subcellular Components: Isolation and Fractionation. Butterworths, London, 1972, pp. 279-310.

Fishbein, L. and Flamm, W. G.: Potential environmental chemical hazards. Part I. Drugs. The Science of the Total Environment. 1:1972. (In Press).

Fishbein, L. and Flamm, W. G.: Potential environmental chemical hazards. Part II. Food additives and pesticides. The Science of the Total Environment. 1:1972. (In Press).

Fishbein, L. and Flamm, W. G.: Potential environmental chemical hazards. Part III. Industrial and miscellaneous agents. The Science of the Total Environment. 1:1972. (In Press).

Flamm, W. G. and Fishbein, L.: Mutagenic agents. Science. 175:980, 1972.

Serial No.: NIEHS-M-003
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Investigation of Normal Semiconservative Replication Patterns of DNA"

PREVIOUS SERIAL NUMBER: NIEHS-CB-015

PRINCIPAL INVESTIGATORS: J. B. Patterson, Ph.D. and W. G. Flamm, Ph.D.

OTHER INVESTIGATORS: N. J. Bernheim

COOPERATING UNITS: None

MAN YEARS

Total:	.3
Professional:	.3
Other:	.0

PROJECT DESCRIPTION

OBJECTIVES: A large number and variety of chemical compounds inhibit DNA synthesis and prevent the attainment of an appropriate G2 or 4n amount of DNA. In contrast, certain other substances, including some alkylating agents, fail to inhibit DNA synthesis but induce the accumulation of a greater than normal amount of DNA in G2 cells. Undoubtedly, other compounds affect the process of replication in even more subtle ways and may lead to lethal or mutagenic events. Efforts to more fully characterize normal replication of the mammalian genome is predicated on the belief that it will help us to better understand the possible mutagenic effects of environmental agents such as alkylating agents and certain antibiotics.

METHODS EMPLOYED: Mouse lymphoma cells (L5178Y) are blocked in metaphase with colcemid and held at the end of G1 with fresh medium containing 5-fluorodeoxyuridine. Following reversal and the beginning of S-phase, DNA replication is assessed by incubating the cultures in medium containing 5-bromodeoxyuridine. DNA is extracted and fractionated by isopycnic centrifugation in CsCl density gradients. Ribosomal RNA (rRNA) can be made highly radioactive and can be isolated essentially free of other RNA classes. Purification is achieved by first isolating clean ribosomes, isolating RNA from them, then separating the RNA's on sucrose density gradients. With these fractions of DNA and rRNA one can perform molecular hybridization experiments to determine the point in time in S-phase at which replication of the ribosomal genes occurs.

MAJOR FINDINGS: The mouse genome replicates in such a fashion that the nucleotide sequences (genes) undergoing synthesis in early S-phase are of a different chemical composition from those genes or nucleotide sequences which replicate in middle or late S-phase. At the beginning of S-phase, only those nucleotide sequences which have a high guanine plus cytosine content undergo replication and, as replication proceeds, the guanine plus cytosine content of replicating sequences gradually decreases. From the beginning to the end of S-phase a change of 10% in guanine plus cytosine content is observed. It now appears that this is a general phenomenon applicable to, perhaps, all mammalian cells (including those of human origin) and, while the underlying mechanism is not understood, these observations show that specific nucleotide sequences replicate at fixed times within S-phase. By introduction of a BUdR-density label into newly replicated DNA, we can identify DNA which has been replicated at any chosen time in S-phase. We have isolated DNA from synchronized cultures in the appropriate time periods (early, mid, and late S-phase) and have separated newly replicated from non-replicated DNA. One class of genes which produce large amounts of an easily identifiable and isolatable product are the ribosomal RNA (rRNA) genes. We have succeeded in isolating ^{32}P- labelled 18S and 28S rRNA. In order to answer the questions we have posed, all that now remains to be done is the hybridization experiments. We have performed preliminary hybridization experiments and find no potential problems that should interfere with the successful completion of this project.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The above study has provided the basis for testing and evaluating the potential of environmental agents in altering normal patterns of DNA replication and may help explain the spectrum of biological and genetic effects produced by these agents.

PROPOSED COURSE: The above study indicates that specific genes are replicated at fixed times within S-phase which should enable us to isolate preparations of DNA which are greatly enriched in certain genes for transformation studies. We are presently attempting to learn when ribosomal and transfer RNA genes are replicated within S-phase.

PUBLICATIONS

Flamm, W. G., Bernheim, N. J., and Brubaker, P. E.: Density gradient analysis of newly replicated DNA from synchronized mouse lymphoma cells. Expt. Cell Research. 64: 97-104, 1971.

Flamm, W. G., Birnstiel, M. L., and Walker, P. M. B.: Isopycnic centrifugation of DNA: methods and application. In Bernie, G. D. (Ed.): Subcellular Components: Isolation and Fractionation. (2nd. edition) London, Butterworth's, 1972, pp. 279-310.

Flamm, W. G.: Highly repetitive sequences of DNA in chromosomes. In J. F. Danielli (Ed.): International Review of Cytology. New York, Academic Press, 32, 1972, pp. 1-51.

Patterson, J. B. and Stafford, D. W.: Sea urchin satellite DNA. Its large-scale isolation and hybridization with homologous rRNA. Biochemistry. 9: 1278, 1970.

Serial No.: NIEHS-M-004
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Mutational Assay Systems Utilizing Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-CB-012

PRINCIPAL INVESTIGATORS: D. Clive, Ph.D. and W. G. Flamm, Ph.D.

OTHER INVESTIGATORS: M. R. Machesko, N. J. Bernheim

COOPERATING UNITS: None

MAN YEARS

Total:	3.0
Professional:	1.5
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: Because of their large numbers, relatively short generation times, biological relevance to mammalian genetics and ease of handling, mammalian cells grown in suspension culture offer a highly sensitive system for assaying the mutagenicity of environmental agents. Elucidation of the mechanism of mutagenicity of such agents requires the establishment of a number of well-defined mutant stocks produced by mutagens with a known mechanism of action. Back-mutation induced by an undefined agent in, say a transition-type mutant but not in an addition-deletion type mutant, would characterize the unknown mutagenic agent as causing transition-type base substitutions. It is our objective to develop the mutant stocks and the proper selection media required for assaying and characterizing the mutagenicity of environmental agents. Inherent in this is the evaluation of the critical parameters (plated cell concentrations, concentration of selective agents, recovery of mutants) affecting the accurate determinations of mutation rate. To achieve these objectives we have focussed our efforts on the development of mutants at the thymidine kinase (TK) locus.

METHODS EMPLOYED: Thymidine kinase- (TK-) deficient mutants of L5178Y mouse lymphoma cells are isolated as resistant colonies growing in soft agar cloning medium supplemented with 50 µg/ml of 5-bromodeoxyuridine (BUdR); this medium selects against TK-competent cells by virtue of their ability to effect a TK-mediated phosphorylation of BUdR which ultimately is lethal. TK-deficient cells are selected against by a medium supplemented with thymidine (T), hypoxanthine (H), methotrexate (M, to innibit folic acid metabolism) and

glycine (G) (THMG medium), whereas TK-competent cells can utilize the exogenous thymidine to overcome the methotrexate block and, hence, survive. Spontaneous mutation rates (forward and reverse) are determined by the rate of mutant accumulation in a culture and, where practicable, by fluctuation test. TK mutant phenotypes are confirmed by determining the rate at which their cell lysates can phosphorylate thymidine (TK assay). The efficiency of mutant recovery in each of the selection media is determined in reconstruction experiments, utilizing standard mutant stocks.

Potential chemical mutagens are assayed as follows: test cells of known genotype are treated with the compound for 2h, the cells are washed, allowed to express genetic lesions for 48h, cloned in selective cloning medium and scored for surviving mutant colony frequency after 7-10 days incubation.

MAJOR FINDINGS: The thymidine kinase (TK) locus in L5178Y mouse lymphoma cells appears diploid and the 3 possible mutant forms -- the $TK^{+/+}$ and $TK^{-/-}$ homozygotes and the $TK^{+/-}$ heterozygote -- have been isolated and characterized as a prerequisite to their use in this mutational assay system. The TK-competent clonal lines ($TK^{+/+}$ and $TK^{+/-}$) clone efficiently in soft agar cloning medium supplemented with THMG but are quantitatively killed when cloned in the presence of BUdR. On the other hand, TK-deficient ($TK^{-/-}$) cells are killed by THMG but are unaffected by BUdR.

TK activities for the $TK^{-/-}$, $TK^{+/+}$ and 9 independently arising $TK^{+/-}$ back mutants are, respectively, 0, 61 and 27.5 ± 1.5 μmoles of thymidine phosphorylated/10^6 cells/minute of incubation.

The $TK^{+/+} \rightarrow TK^{-/-}$, $TK^{-/-} \rightarrow TK^{+/-}$ and $TK^{+/-} \rightarrow TK^{-/-}$ spontaneous mutation rates are, respectively $\sim 5 \times 10^{-11}$ (based on recovery of only 1 mutant), 6.4×10^{-9} and 1.2×10^{-7} mutations/locus/generation.

Reconstruction experiments have shown the recovery of nearly 100% of added $TK^{-/-}$ mutant cells when up to 8×10^4 $TK^{+/+}$ cells are plated per ml of BUdR-supplemented cloning medium; similar recoveries are obtained by co-plating $TK^{+/+}$ cells in the presence of up to 1×10^5 $TK^{-/-}$ cells per ml of THMG-supplemented cloning medium.

The forward ($TK^{+/-} \rightarrow TK^{-/-}$) mutation rate has been greatly stimulated using known mutagens (X-irradiation, ethyl methanesulfonate) and a suspected mutagen (hycanthone methanesulfonate -- see Project No. NIEHS-M-006), thus demonstrating the efficacy of this system in mutagen detection.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is integrated with the development of a mouse-mouse host-mediated assay for chemical mutagens (Project No. NIEHS-M-005) and with studies on the mutagenicity of environmental agents (Project No. NIEHS-M-006) and shares their significance. Further, this *in vitro* system permits both quantitative and qualitative (i.e., mechanistic) evaluation of a chemical's mutagenic effects on the mammalian genome. By studying forward and back-mutation rates at the TK locus, one can characterize a compound as being, for example, a mildly potent, addition- or deletion-type mutagen. In conjunction with the host-mediated assay, it should be possible to distinguish between mutagenicity of the parent compound and of its metabolite(s).

PROPOSED COURSE: Since the TK locus is present in diploid condition, the usual techniques for the development of specific mutations (e.g. transitions, transversions, frame-shifts) at this locus (so-called tester strains) must be modified to effect the same type of mutation at both alleles. Using "clean" mutagens -- i.e., mutagens which produce predominately one type of mutation -- we plan on producing, for example, a diploid frame-shift tester strain, by the process of frame-shifting one allele, back-mutating the second allele (with a non-frame-shifting mutagen), then frame-shifting the second allele. The resulting, $TK^{-/-}$ should be a double frame-shift mutant capable of back-mutating only when exposed to a frame-shift mutagen. $TK^{-/-}$ mutants of transition or transversion type might also be produced in similar manner. An uncharacterized environmental mutagen can then be defined by determining which type of mutant it is capable of back-mutating.

PUBLICATIONS

Clive, D., Flamm, W. G., Machesko, M. R., and Bernheim, N. J.: A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Res. (In Press).

Clive, D., Flamm, W. G., and Patterson, J. B.: Specific locus mutational assay systems for mouse lymphoma cells. In Hollaender, A. (Ed.): Environmental Chemical Mutagens. v.3, Plenum, New York-London, (In Press).

Serial No.: NIEHS-M-005
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "A Mouse-Mouse Host-Mediated Assay for Chemical Mutagens"

PREVIOUS SERIAL NUMBER: NIEHS-CB-013

PRINCIPAL INVESTIGATORS: D. Clive, Ph.D. and W. G. Flamm, Ph.D.

OTHER INVESTIGATORS: M. R. Machesko

COOPERATING UNITS: None

MAN YEARS

Total:	.2
Professional:	.1
Other:	.1

PROJECT DESCRIPTION

OBJECTIVES: Some chemicals have been shown to exhibit species-specificity in their mutagenic activity. Such variation has been attributed to differences in host metabolism of the compound (converting non-mutagens to mutagens or vice versa), or to differences in chromosomal architecture between, say, bacteria and mammals. The ideal mutagen assay system should combine the operational ease and high sensitivity of bacterial test systems with the biological relevance of expensive, time-consuming whole mammal test systems. This system should, in effect, expose a large number of mammalian genomes to the test compound and its products of mammalian metabolism, in a mammalian milieu. We intend to use mouse lymphoma cells (the mammalian genetic indicator) implanted within various mouse body cavities or in general circulation (the mammalian metabolism and milieux) to realize these ideals.

METHODS EMPLOYED: L5178Y mouse lymphoma cells, either heterozygotic or deficient at the thymidine kinase (TK) locus ($TK^{+/-}$ or $TK^{-/-}$ genotype, respectively), are implanted within a DBA/2J mouse. Implantation sites presently under consideration include the peritoneal cavity and the circulatory system. After the cells have achieved exponential growth (ca. 3d post inoculum) a known or suspected mutagen is administered by various routes. After 1-4 days incubation within the mouse the cells are removed, diluted into culture medium and allowed to stand without agitation for 24 hours. During this time cells other than the test cells either lyse or attach to the container surface. The test cells are removed, then cloned for mutant frequency and viability (for description of cloning and mutagen assay techniques see Project No. NIEHS-M-004).

MAJOR FINDINGS: Actual implementation of the mouse-mouse host-mediated assay is dependent upon the development and testing of the *in vitro* test system, (described in detail under Project No. NIEHS-M-004). Now that this phase has advanced to the point where the assay can detect mutagens not previously examined in mammalian systems (see Project No. NIEHS-M-006), extension of the assay to host-mediated studies is possible. Preliminary attempts at growing our L5178Y cells in the mouse (after having been cultured *in vitro* for 3 years) indicate the need for some adaptation of our mutant stocks to *in vivo* growth as a prerequisite to full-scale work on the development of this system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During the past century man has seen the creation and dispersal of an unknown number of actual and potential mutagens, physical, chemical and biological. If we are to avoid the generally agreed-upon long-term disastrous effects of rapidly accumulated mutations within any biological population we urgently need methods which aid in the detection and control of mutagens. Test systems for the detection of mutagens exist; each of them has limitations in terms of sensitivity, ease of operation and biological relevance to man and other economically important species. The mouse-mouse host-mediated assay for chemical mutagens which has been described above, combines operational convenience and high sensitivity with biological relevance to mammals.

PROPOSED COURSE: We plan to make this assay operational using as a criterion the stimulation of mutation frequency by known mutagens administered via various routes. Later, environmentally significant chemicals (e.g. DDT, food additives such as nitrites) will be tested for their mutagenicity in like fashion.

Serial No.: NIEHS-M-006
1. Mutagenesis Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Detection of Environmental Mutagens"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: D. Clive, Ph.D. and W. G. Flamm, Ph.D.

OTHER INVESTIGATORS: M. R. Machesko

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The rapid increase in both variety and quantity of environmental mutagens is intimately associated with attempts to "better living through chemistry". It is presently possible to partake of these compounds via ingestion, inhalation and injection (e.g., nitrites and certain other food additives; TMP in automotive exhausts; and certain chemotherapeutic agents). By identifying specific compounds as mutagens it is hoped that a more cautious approach to their use will minimize the increased frequency of serious genetic disorders that exposure to such agents is expected to produce in subsequent generations.

We have developed an *in vitro* mutagen assay (see Project No. NIEHS-M-004) utilizing thymidine kinase heterozygote ($TK^{+/-}$) L5178Y mouse lymphoma cells, which responds quite sensitively to treatment with known mutagens. As a beginning for our screening program for environmental mutagens we have examined the drug hycanthone methanesulfonate (Etrenol, Winthrop), widely used in the tropics as an antischistosomal agent, and reported by Hartman *et al* to be mutagenic in bacteria.

METHODS EMPLOYED: (These are described in detail under Project No. NIEHS-M-004). Briefly, the mutagen assay involves exposing a culture of $TK^{+/-}$ L5178Y mouse lymphoma cells to the test compound, washing the cells, and allowing them to express any genetic lesions that might have been introduced. An identical culture is sham treated as a control. Following expression time the cells are plated in soft-agar cloning medium supplemented with 5-bromodeoxyuridine (BUdR); subsequent incubation kills off all but the $TK^{-/-}$ mutant cells which

form visible colonies after 7-10 days. Viable counts, reconstruction using stock $TK^{-/-}$ mutants and positive (EMS) controls permit us to evaluate quantitatively the extent to which the given treatment has enchanced the mutation rate of these cells.

MAJOR FINDINGS: Classical mutagens have been used to correlate the response of our system with mutagenic hazard in whole animals. Thus, 600 R of X-irradiation stimulates the $TK^{+/-} \rightarrow TK^{-/-}$ mutation rate 2500-fold over the spontaneous rate. The corresponding figures for the potent alkylating agent ethyl methanesulfonate (EMS, 10^{-2}M and 10^{-3}M) are 17,000-fold and 900-fold, respectively. Surprisingly, similar treatment with a popular antischistosomal drug, hycanthone methanesulfonate (10^{-4}M), resulted in a 450-fold stimulation of the mutation rate. This suggests that hycanthone methanesulfonate is about 5 times as mutagenic as the recognized classical mutagen, EMS.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During the past century man has experienced the creation and dispersal of an unknown number of actual and potential mutagens, physical, chemical and biological. The genetic consequences of human exposure to such agents may range from severe first-generation effects to a gradual debilitation of the species over tens of generations. An alternative to such retrospective recognition of a mutagenic hazard is, of course, the early detection and control of environmental mutagens. Essential to such detection is a safety testing program utilizing a simple, sensitive mutational assay system, such as the one used here (see also Project Nos. NIEHS-M-004 and NIEHS-M-005). The demonstration of the mutagenicity of hycanthone in mammalian cells has given additional impetus to safety testing at NIEHS.

PROPOSED COURSE: Continued investigation of the mutagenicity of select compounds using the $TK^{+/-} \rightarrow TK^{-/-}$ *in vitro* system will be augmented by more refined techniques as they become available. These will include the mouse-mouse host-mediated assay (see Project No. NIEHS-M-005) and the use of special tester strains in a $TK^{-/-} \rightarrow TK^{+/-}$ mutational assay system, for the better characterization of confirmed mutagens (see Project No. NIEHS-M-004).

PUBLICATIONS

Clive, D., Flamm, W. G. and Machesko, M. R.: Mutagenicity of hycanthone in mammalian cells. Mutation Res. 14: 262-264, 1972.

PATHOLOGIC PHYSIOLOGY BRANCH

PATHOLOGIC PHYSIOLOGY BRANCH
Summary Statement

The Pathologic Physiology Branch is fortunate to have recently had a number of scientists join it who were formerly with the Cell Biology Branch. In addition, five other investigators have been hired during the past two months to support the mission of the Pathologic Physiology Branch which continues to be two-fold: (1) solid basic biologic research into the mechanisms of the toxic effects of environmental agents, and (2) a histopathologic and photographic service function in support of the Institute. Scientists in the Branch are trained in biochemistry, pharmacology, and/or pathology, and this broad base of expertise is reflected in the diverse research projects reported individually in this Annual Report.

Dr. William Jurgelsky, with support from the Animal Science and Technology Branch, has continued to develop the opossum as a Marsupial model system which will be useful in the investigation of a large variety of physiologic, pharmacologic, pathologic and toxicologic mechanisms. The Marsupial is at the inframammalian position in the evolutionary scale, and is endowed with a number of unique developmental characteristics which include (1) a gestation period of 12-3/4 days; (2) no placenta; (3) intrapouch development as an extension of intrauterine development; (4) markedly delayed development of the nervous system and respiratory system; (5) "simplified" thyroid, pituitary and brain; (6) anomalous iodine transport and storage; (7) extreme tolerance of developing young to high CO_2 levels; and (8) a markedly increased growth rate during the first year of life. All of these characteristics account for the uniqueness of this model system and present many useful areas for the study of environmental hazards. These animals are being utilized experimentally not only by members of the Pathologic Physiology Branch but, when available, by the Institute in general. Dr. Jurgelsky has continued a variety of investigations which include the potential uses of the Marsupial neonate as a model for the identification and evaluation of environmental toxicants; an evaluation of the toxicity of sex hormones to the opossum; chemical and morphologic studies of the role of lipid metabolism in CNS irritability and seizures; and the effects of excess iodine and iodine deficiency on the endocrine system of the opossum. This opossum colony is obviously a valuable asset to the Institute.

Dr. Robert Goyer from the Department of Pathology at the University of North Carolina Medical School spent the past year with the Pathologic Physiology Branch. Dr. Goyer's presence and expertise in pathology has accounted for many valuable contributions to the Institute in addition to his fine research efforts. He has continued to investigate the pathogenesis of heavy metals, and has attempted to identify the toxic cellular effects of heavy metals, particularly the adaptation of detoxifying mechanisms and the other metabolic factors which influence pathologic effects. In addition, Dr. Goyer has

studied the toxicity of orally ingested NTA, a chelating agent which is proposed as a phosphate replacement in the manufacture of detergents. Dr. Goyer has described a hyperglycemic effect in rats and also characteristic vacuolar lesions occurring in the renal proximal tubules of NTA treated animals. It is hoped that Dr. Goyer will retain a close working relationship with the Institute in the coming year.

Dr. Herbert Posner has continued his studies of the factors affecting the placental passage of various environmental agents and investigations of the biologic distribution of various chemicals with special emphasis on the fetus. In addition, studies of the relative toxicity of exogenous compounds as a function of age are also being extended. The mechansims by which toxic substances reach the fetus, and the mechanisms or factors which control or alter placental penetration of foreign chemicals, are obviously important to the general area of environmental health.

Doctors Klein and Herman have continued a large variety of studies in an attempt to assess many of the potential toxicologic hazards associated with methylmercury. These investigations include: (1) a comparison of conditioning behavior in rats treated in utero with methylmercury and non-treated rats; (2) the effects of methylmercury exposure via the milk in nursing neonate rats; (3) the morphologic lesions in rat kidneys associated with acute and chronic methylmercury poisoning; (4) the general pathologic effects of methylmercury intoxication in rats; (5) the comparative pathology of acute methylmercury intoxication in guinea pigs; (6) an evaluation of the potential antidotal effect of glutamate therapy in methylmercury poisoning in adult male rats; (7) the neuropathology of subacute exposure to methylmercury; (8) the metabolism of methylmercury hydroxide by the nervous system; (9) the function and pathology of methylmercury hydroxide relative to the central nervous system; and (10) an assessment of the neurotoxicity with pathologic and physiologic correlation of methylmercury in adult rats. This large variety of studies is of definite value to the overall understanding of methylmercury intoxication.

Dr. Judson Spalding has continued his studies involving mammalian cell cultures and the effects of environmental toxicants on these in vitro cells. He has developed a sensitive, semi-micro assay system for measuring the toxicity of purified mycotoxins in the cell culture system. In addition, he is currently investigating the mechanisms of the induction of resistance to pesticides in cultured lymphoma cells. Cultured cells provide another valuable tool to the Pathologic Physiology Branch for the evaluation of the toxic effects of environmental agents and for the further study of biochemical and physiological mechanisms of intoxication.

Dr. George Lucier has been primarily concerned with the mechanisms of hepatic microsomal drug metabolism and the effects of environmental chemicals on the metabolism of foreign and endogenous substrates. His studies include an assessment of the effects of heavy metals on hepatic microsomal enzymes; the alterations in liver microsomal and lysomal β-glucuronidase by compounds which induce drug metabolizing enzymes; the metabolism of some carbamate pesticides by liver, kidney, lungs, stomach, intestines and blood; the effect of chlordane and methylmercury on the metabolism of carbamate pesticides; the purification

and determination of enzymic activity of microsomal liver UDP-glucuronyl-transferase following treatment with piperonyl butoxide; and the effects of methylmercury on serum and brain cholinesterase activity. Dr. Lucier's laboratory has been exceptionally productive. This fact is reflected in his numerous publications this year.

Dr. B. Dean Nelson has continued his studies of the mechanisms of mitochondrial electron transport and the effects of environmental chemicals on cell respiration during the past year. He has expanded his investigation on the role of coenzyme Q and cytochrome B in oxidative phosphorylation and the effect of environmental agents such as chlordane on the biochemistry and biogenesis of mitochondria. This laboratory is another good example of the blending of outstanding basic research in biochemistry with an application of similar research techniques to other more immediately relevant questions concerning environmental toxicology.

A number of new project areas have just been initiated during the past month. These studies include the investigation of mechanisms of drug secretion (renal and biliary) and the effect of environmental agents on such processes; the biochemical mechanisms of spermatogenesis and the toxic effect of exogenous chemicals on male reproduction; the biochemical mechanisms associated with oogenesis and the toxic effect of exogenous chemicals on female reproduction; the effect of prenatal exposure to chemicals on the subsequent reproductive performance of the offspring; and the carcinogenic effect of prenatal exposure to environmental chemicals.

As already mentioned, the Pathologic Physiology Branch plays a major service role at the Institute by providing histopathological and photographic support to individual scientists. The histology laboratory aids in necropsy, prepares tissue for light microscopy and autoradiography. During the past fiscal year, the laboratory has processed and sectioned 22,753 blocks of tissue to yield 23,000 H&E stains, 1,647 special stains, and 248 autoradiograms. In addition, 723 blocks of tissue were contracted to a private laboratory. Interpretation of histopathologic tissue changes is provided by Dr. Bill Bullock and Dr. Anna Ottolenghi, consultants to the Branch for routine histopathology. The electron microscopy area provides ultrastructural support to the Institute. Tissues submitted are processed, sectioned, and reviewed microscopically. Subcellular areas of interest are photographed for interpretation. During the past year the laboratory has processed and embedded 1,066 blocks of tissue to yield 462 thick sections and 307 thin sections; 2,179 photographs were provided for interpretation.

Special acknowledgement should go to Mr. Fred Talley and his fine group of coworkers for their efforts in the histology support function of the Branch. As documented above, Mr. Talley's group is especially efficient and productive.

The photography laboratory is responsible for photographic services to the Institute. In addition to special projects, the laboratory has prepared 4,750 black and white negatives; 1,910 black and white transparencies; 2,344 color transparencies; 10,567 black and white prints; 136 color prints; and 2,650 feet of movie footage during the past fiscal year.

The Pathologic Physiology Branch will continue to undergo some administrative and organizational changes during the coming year as a result of the recent increase in personnel. Changes will be necessary to more effectively carry out the charge of the Institute which, stated broadly, is to provide information to assess and understand the toxic potential of environmental agents.

Serial No.: NIEHS-PP-002
1. Pathologic Physiology Branch
2. Cellular Control and Morphogenesis Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effects of Excess Iodine and Iodine Deficiency on the Endocrine System of the Opossum (Didelphys marsupialis virginiana Kerr)"

PREVIOUS SERIAL NUMBER: NIEHS-PP-102

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Paul J. Davis, M.D., Baltimore City Hospitals, and Pearlie M. Hudson

COOPERATING UNIT: Endocrinology Division, Baltimore City Hospitals, Baltimore, Maryland

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Work performed elsewhere by members of this laboratory has revealed that the adult opossum develops a flagrant intrafollicular thyroiditis in response to the administration of potassium iodide at levels within the therapeutic range of humans. The implication from these studies, as well as from those of other workers who have demonstrated a low PBI in the opossum and a low thyroxine secretion rate, is that the marsupial has an iodine transport and storage mechanism that differs markedly from that of other mammals and is thus worthy of evaluation as a potential model for studies of the role of iodine in the endocrine system. The objective of these studies is to define the structural and chemical changes that take place within the hypothalamic-pituitary-thyroid axis of the opossum in response to iodine excess and iodine deficiency.

METHODS EMPLOYED: Through the use of column chromatography, thin-layer chromatography and scintillation spectroscopy, in vivo and in vitro radioiodine metabolism is being studied in adult opossums treated with potassium iodide. Histological changes are being documented by light and electron microscopy.

MAJOR FINDINGS: Completion of a large scale toxicity study has failed to confirm the earlier reported apparent induction of goiter by excess iodine in the opossum. This experiment implied that while opossums, unlike other species, do not escape the Wolff-Chaikoff effect (organic binding of iodine remains suppressed), compensatory changes in thyroid function do not lead to hypertrophy. The mechanism remains unclear at present. While thyroiditis develops in response to high KI administration, the lesion is inconsistent. However, biliary cirrhosis is a frequent finding in KI treated animals. Biliary cirrhosis has not been reported as the result of KI administration in other species. An experiment which will permit the study of the time course of the thyroid and liver lesions and the effect of prolonged excess iodide ingestion of thyroid hormone levels is underway.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The intricacies of the role of iodine in the mammalian endocrine system are largely unknown. Such knowledge is essential to an understanding of many endocrinopathies, including thyroiditis, hyperthyroidism, and hypothyroidism and for evaluation of the possible role of environmental contaminants in these pathologic processes. Study of iodine metabolism in an animal such as the opossum in which response to iodine appears anomalous could yield insight into mechanisms of iodination, iodine transport, and iodine storage.

PROPOSED COURSE: The effect of excess KI on thyroid hormone levels will be explored, and the effect of thyroid ablation on development of the liver lesion will be studied.

Serial No.: NIEHS-PP-003
1. Pathologic Physiology Branch
2. Cellular Control and Morphogenesis Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Chemical and Morphologic Studies of the Role of Lipid Metabolism in CNS Irritability"

PREVIOUS SERIAL NUMBER: NIEHS-PP-103

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Pearlie M. Hudson, Clyde Boyd, Frederick Snyder, M.D., NIMH, and Michael O'Connor, NINDS

COOPERATING UNITS: Section on Electroencephalography, NINDS
Laboratory of Psychobiology, NIMH

MAN YEARS

Total:	1.0
Professional:	0.2
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: Disorders of steroid metabolism have been implicated among the causes of epilepsy. Previous studies, performed elsewhere by members of this laboratory, have established that an androgen-related, cholesterol-biosynthesis inhibitor, when administered once weekly to young marsupials (Didelphys marsupialis virginiana Kerr) in doses well below the toxic level, will induce a chronic major motor seizure state. Seizures may be induced at will by tactile stimulation. The opossum alone, among those standard laboratory species thus far tested, develops CNS hyperirritability in response to this drug. A number of organic phosphates and chlorinated hydrocarbon pesticides, which are known to influence steroid metabolism, also produce seizures at high levels of exposure. In the studies underway alterations in lipid metabolism produced by this drug and other agents, such as pesticides with seizure-producing potential, will be correlated with changes in CNS irritability.

METHODS EMPLOYED: Optical microscopy, electron microscopy, thin-layer chromatography, electroencephalography, ultra centrifugation, gas chromatography and scintillation spectroscopy are being used. Comparative studies are being performed in rats and opossums.

MAJOR FINDINGS: Comparative drug studies have revealed that neither triparanol, a non-steroid cholesterol biosynthesis inhibitor (which, like U18666A the steroid-like seizure inducing drug, blocks cholesterol synthesis terminally to cause desmosterol accumulation), nor clofibrate, a drug which blocks cholesterol synthesis prior to squalene without accumulation of desmosterol, induce seizures in the opossum. This finding is similar to that of Winer and others who have shown that the steroid configuration as well as accumulation of desmosterol were required for the induction of myotonia with cholesterol biosynthesis inhibitors in the rat. Contrary to the work of Winer, the opossum does not develop myotonia, nor have we been able to demonstrate a protective effect of a high cholesterol diet. This latter is not surprising in view of the known in situ cholesterol biosynthesis in brain and the high blood brain barrier to cholesterol.

Clofibrate produces negligible effects on total brain sterol composition, while triparanol lowers cholesterol levels by about 30 percent compared to a 70 percent lowering by the steroid-like seizure inducing drug. Absolute cholesterol levels are three times lower in U18666A treated animals than in triparanol treated animals.

Relative to litter mate controls, there is a 10-fold higher ratio of brain desmosterol formed in triparanol treated than in the seizure inducing drug treated animals. However, the whole brain desmosterol to cholesterol ratio is 10 times higher in the animals receiving the seizure inducing drug.

Preliminary electroencephalographic studies indicate that spontaneous seizures in animals receiving the seizure inducing cholesterol biosynthesis inhibitor are generalized, limited to the brain and occur predominantly in the early morning hours beginning during apparent REM sleep.

There appears to be a definite correlation between age at initial drug exposure and seizure incidence and type. Animals first treated prior to 3 months of age develop clonic tonic seizures; those exposed later have either less frequent seizures or have seizures limited to the clonic phase. The large interlitter and intralitter variation in seizure inducibility implies a genetic basis for response to the drug.

An unexpected finding was the development of a malabsorption syndrome associated with ricketts as the result of triparanol administration. Malabsorption attributed to ingestion of triparanol has been reported in humans and rats, but in the opossum there is much less correlation between degree of mucosal dmage and clinical evidence of malabsorption implying a somewhat different mechanism than in the other species evaluated.

Portions of these data have been presented at the 85th Annual Meeting of the American Association of Anatomists and at the 56th Annual FASEB Meeting.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The induction of seizures in the marsupial by administration of an inhibitor of cholesterol biosynthesis represents a previously unreported simply

reproducible model for experimental epilepsy. The apparent direct implication of disordered steroid biosynthesis in the seizure state in these animals may offer a new window on neural irritability in general, and on epilepsy in particular.

PROPOSED COURSE: Further attempts will be made to determine the extent to which alterations in sterol metabolism are specifically associated with the administration of the androgen related drug. Toward this end, studies with the tagged drug are planned to determine its neural metabolism and localization. Comparative chemical studies of drug treated litter mates with and without seizures will be performed in an effort to correlate degree of sterol alteration with degree of neural hyperirritability.

Further electroencephalographic studies are in progress to explore the correlation between EEG and tactile induction and between REM sleep and seizures.

A small panel of pesticides with known epileptogenic potential will be tested in the opossum for seizure induction.

Serial No.: NIEHS-PP-004
1. Pathologic Physiology Branch
2. Cellular Control and Morphogenesis Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants"

PREVIOUS SERIAL NUMBER: NIEHS-PP-104

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Ellen D. Porter, Clyde W. Boyd, Pearlie M. Hudson, Alfred Travis, and H. B. Matthews, Ph.D., NIEHS
Gerald Alderson, D.D.S., M.D., AFIP
Wilna Wood, Ph.D., Judith Massicot, Clyde Dawe, M.D., and Michael Chirigos, Ph.D., NCI
Lorenz Zimmerman, M.D., K. Ishak, M.D., F. Mostofi, M.D., and L. Hockholder, M.D., AFIP
Arthur Moore, Paul Mortillaro, Jack Romine, NIH
U. J. Lewis, Ph.D., Scripps Institute, LaJolla, Calif.

COOPERATING UNITS: Animal Science and Technology Branch, NIEHS
Laboratory of Pathology, NCI
Viral Biology Branch, NCI
Ophthalmologic, Heptic, Renal, Pulmonary and Oral Pathology Branches, AFIP
Motion Picutre Section, NIH
Analytical and Synthetic Chemistry Branch, NIEHS
Animal Resources Department, Duke University

MAN YEARS

Total:	6.0
Professional:	3.0
Other	3.0

PROJECT DESCRIPTION

OBJECTIVES: The inframammalian position of the marsupial in the evolutionary scale endows the animal with characteristics not present in any other mammal. Some of these attributes are: (1) gestation period of 12-3/4 days; (2) no placenta, (3) intra-pouch development as an extension of intrauterine development, permitting experimental manipulation of young at birth at a stage of development comparable to that in utero in other mammals; i.e., equivalent to an eight-week-old human and eleven-day-old rat in utero;

(4) markedly slow development of the nervous and respiratory systems and hind limbs; (5) "simplified" pituitary, thyroid and brain; (6) anomalous iodine transport and storage; (7) extreme tolerance of developing young to high CO_2 levels; and (8) marked growth rate during first year with a newborn to adult weight ratio of 1:40,000 (compared to 1:40 for the rat). These characteristics, many of which would appear to provide the basis for unique model systems for the study of environmental hazards, have not been exploited experimentally. With this objective, a colony of indigenous opossum (Didelphys marsupialis virginiana Kerr) has been established; and a preliminary evaluation of response of the neonatal opossum to selected toxicants is underway.

METHODS EMPLOYED: Special pharmacological techniques, light and electron microscopy, ultra centrifugation, thin-layer chromatography and gas chromatography are being used.

MAJOR FINDINGS: A total of 105 litters of opossums were produced from the breeding colony established in the 1970-1971 season. Thus far in the 1971-1972 season, a total of 90 litters have been born in the first breeding period representing an 80% conception rate. These results establish the efficacy of the facility design and the controlled breeding techniques developed during the past two years.

A closed colony established by Animal Science and Technology Branch with the objective of supplying more uniform domestic opossums to the program is now in the second generation. Conception rate in this group bred without knowledge of estrus is 65% despite losses of males from fighting.

A simple quantitative technique has been developed for the parenteral administration to the newborn opossum of test substances in solution. The lack of such a procedure (use of standard syringes and needles is not feasible due to body size and mobility of the young) has been a major handicap to the exploitation of the possibilities offered by the opossum neonate for pharmacologic-toxicologic-carcinogenesis studies.

A description of the techniques which have been developed for manipulating the opossum and details of the facility design are being prepared for inclusion as several chapters in the series, Biology of the Marsupial, to be published by Academic Press. These data are also in press for publication in appropriate journals and will be presented at the 23rd Annual Session of the American Association for Laboratory Animal Science.

A motion picture of the marsupial as a bio-medical model being produced in collaboration with the Motion Picture Unit of the Medical Arts and Photography Branch, NIH, is approximately one-third completed. The movie will be in two parts and will describe, respectively, the adult and the neonate opossum as laboratory models. Projected release date is Spring of 1973.

A definitive study of ethyl nitrosourea (ENU) carcinogenesis in the opossum is in progress. Animals representing 11 different age groups were given a single oral dose of 100 mg/kg ENU one year ago. Thus far, the following tumors have been produced (order indicates relative incidence): odontogenic

tumors of the jaw, nephroblastoma, hepatoma, biliary carcinoma; glial tumors of the brain and spinal cord; adenocarcinoma of the glandular stomach; fibrosarcoma of the liver, pinna and tongue; hamartomas of the liver and lung; and a teratoid meduloepithehoma of the eye. The latter is the first reported tumor of its kind to be induced in a laboratory animal and is of interest because of its similarity to the retinoblastoma, a tumor which has not been reported in species other than humans. The several embryonic tumors (hamartomas, teratomas) are of interest because of the difficulty of inducing these types of tumors in the usual experimental animal. Portions of these data have been presented at the Annual Meeting of the Cancer Research Society, 1972.

Several of these tumors have been established in tissue culture; many of the cell types are unique in their behavior in vitro. Thus far we have had no success in re-establishing tissue culture cell lines in the opossum.

Experiments initiated this year include attempts to compare the effect of intraperitoneal and intraoccular administration with oral administration of ENU to determine whether the odontogenic and occular tumors are due to systemic or local insult. Preliminary studies are also underway to test marsupial susceptibility to three murine oncogenic RNA viruses and a murine oncogenic DNA virus. Experiments to test the opossum neonate for tolerance to the growth of innoculated human tumor cells are underway using a panel of human tumor cell lines grown in tissue culture.

Collaborative studies on the ontogeny of hepatic enzyme induction in the developing opossum and on the functional development of the pituitary are also underway.

Anatomic studies are in progress with the objective of preparing a light and electron microscopic atlas of the major tissues in the developing opossum. Emphasis is on the endocrine and respiratory systems.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marsupial neonate is, in many respects, a fetus that completes the final portion of its development in an extra-uterine environment. Only in the marsupial is it possible to expose embryonic tissue in vivo to toxic insult without maternal metabolic or placental intervention and without effect of the mother's own metabolism or the trauma of caesarian section. A systematic survey of the response of the marsupial to drugs and toxins with known pharmacologic or carcinogenic activity is not available. Such a study should indicate the value of the newborn marsupial for neonatal toxicology and experimental carcinogenesis and may yield new insight into the response of embryonic tissue to toxic insult. Preliminary findings indicate that the animal may have unique value as a model for studying mechanisms of carcinogenesis and endocrine development.

PROPOSED COURSE: Evaluation of the response of the opossum neonate at the chemical and morphologic level to drugs and toxins of known pharmacological and/or carcinogenic potential will be continued with emphasis on carcinogenesis. Studies of mechanism of carcinogenesis as influenced by organ and

tissue maturity and hormonal and immunologic status will be explored. Further attempts to evaluate the opossum model for use in viral oncogenesis and as a system for growth of human tumor cell lines will be continued.

The feasibility of developing a life support system to permit the maintenance and growth of the opossum young outside the mother's pouch will be explored. The practicability of artificial induction of parturition to obtain completely naive, premature young will also be evaluated.

PUBLICATIONS

Jurgelski, William Jr.: Administration of test materials to the neonatal North American opossum (Didelphys marsupialis virginiana Kerr). Laboratory Animal Sciences, Vol. 21, No. 5, 1971.

Serial No.: NIEHS-PP-005
1. Pathologic Physiology Branch
2. Cellular Control and Morphogenesis Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Toxicity of Sex Hormones to the Opossum (Didelphys marsupialis virginiana Kerr)"

PREVIOUS SERIAL NUMBER: NIEHS-PP-105

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Pearlie M. Hudson, Clyde W. Boyd, and David Rowlands, M.D., University of Pennsylvania

COOPERATING UNITS: Pathology Department, Veterans Administration Hospital, Durham, North Carolina
Pathology Department, University of Pennsylvania Medical School, Philadelphia, Pennsylvania

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: Attempts to improve reproduction performance in the female opossum through the use of serum and pituitary gonadotropins to induce estrus, and estrogen and progesterone to insure receptivity to the male have resulted in a syndrome characterized by anorexia, elevated blood urea nitrogen, elevated serum potassium, gastric ulcers and death within three weeks post treatment. Preliminary work indicates that the syndrome can be reproduced with estrogen alone. The response of the opossum to estrogen is apparently unique among mammalian species and implied either a supersensitivity or an inefficient or inadequate detoxification mechanism. The objective of this study is to define the mechanism of estrogen induced injury in the opossum.

METHODS EMPLOYED: Histologic study at the light and electron microscopic levels, clinical chemistry, hypophysectomy, oophorectomy and adrenalectomy are being employed.

MAJOR FINDINGS: A preliminary study reveals no modification in the immunologic system by estradiol, gonadotropin and progesterone alone and in combination as determined by antibody titer to bacteriophage F_2 virus administered prior to

estrogen treatment; no lymph node alterations were evident in these animals. A definitive toxicity study has been completed and histopathologic evaluation is in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elucidation of the toxic and physiologic mechanisms of action of mammalian hormones is at an early stage. Further knowledge of the physiology and patho-physiology of the estrogens is particularly crucial in view of the large segment of the human female population currently exposed chronically to estrogen derivatives for contraceptive purposes and acutely for treatment of infertility and menstrual dysfunction. A study of the atypical response of the opossum to estrogen may give insight into both normal and abnormal estrogen metabolism and function in eutherian mammals.

PROPOSED COURSE: The metabolism of estrogen in the opossum will be studied using isotope techniques. An attempt will be made to reproduce the syndrome in the absence of several possible target organs, i.e., pituitary, adrenals and ovaries.

Serial No.: NIEHS-PP-006
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Pathology of Acute Methylmercury Intoxication in Rats"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: Ronald Klein, M.D., and Sheldon Herman, M.D.

OTHER INVESTIGATORS: Martin Krigman, M.D., Consultant, University of North Carolina at Chapel Hill, Department of Pathology

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.3
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The object of these experiments was to develop a highly reproducible model of acute methylmercury intoxication in the rat which would be used for further studies.

METHODS EMPLOYED: Adult male Charles River C-D strain rats received from two to seven daily subcutaneous injections of aqueous solution of methylmercury hydroxide, 10 mg Hg/kg body weight. BUN, complete blood count, liver enzymes, tissue proteins, total mercury concentrations and light and EM microscopy of tissues were performed.

MAJOR FINDINGS: Reproducible clinical changes consisting of weight-loss followed by decreased activity, ataxia, weakness, and hind-limb crossing phenomenon were observed.

Biochemical Findings: (1) Normal HCT, WBC count and differential; (2) elevated blood urea nitrogen, normal serum creatinine; (3) early depression of serum protein with a reversal of albumin-globulin ratio with other changes observed after serum protein electrophoresis; (4) elevated SGOT and SGPT; (5) depression of serum cholinesterase activity in females only; and (6) highest concentration of total mercury found in kidneys followed by spleen and liver; central nervous system tissue showed a gradual increase.

In the mercury treated rats, histological examination of the central nervous system revealed an advanced peripheral neuropathy, vacuolization and focal neuronophagia of the anterior horn cells and decreased neuron density in selective brain stem nuclei and pyknotic neurons in the internal granular layer of the cerebellum. Kidney revealed distal and proximal tubule lesions. Atrophy of Malphigian corpuscles and extramedullary hematopores was seen in the spleen. Liver sections revealed an increased number of nucleoli per hepatocyte nucleus. None of these changes were seen in control animals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The model is highly reproducible and is useful in evaluating various therapeutic regimes and metabolic interactions. It has focussed attention on the early changes in both kidney and liver, helping to further explain the early pathogenesis of acute methylmercury intoxication following parenteral administration in the rat.

PROPOSED COURSE: Long term biochemical and histopathological changes in rats given an acute course of methylmercury are in progress in order to determine the reversibility of the lesions.

Serial No.: NIEHS-PP-007
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Neurotoxicity of Methylmercury Hydroxide in Adult Male Rats: Pathologic and Physiologic Correlation"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: Sheldon P. Herman, M.D., and Ronald Klein, M.D.

OTHER INVESTIGATORS: George Somjen, M.D., Ph.D., Consultant, Duke University, Professor of Physiology and Pharmacology; Martin Krigman, M.D., Consultant, University of North Carolina at Chapel Hill, Neuropathologist; and Fred Talley

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The present study, nearing completion, has characterized the EM and light microscopic pathologic changes in rat peripheral nervous system after subacute exposure of rats to methylmercury hydroxide (Me-Hg) administered parenterally. Pathologic alterations in nerve, roots and ganglia were correlated with electrophysiologic properties of these structures throughout various stages of intoxication. Recordings of direct nerve conduction velocity, amplitudes of compound action potentials, electromyograms, twitch tensions and intra-ganglionic cell potentials by Dr. George Somjen have complemented the pathologic observations of Dr. Martin Krigman.

MAJOR FINDINGS: With data soon to be submitted for publication, but with some experiments still in progress, we have found that Me-Hg produces a primary sensory neuropathy in the rat. Neuropathy progresses over time and precedes CNS involvement. In vivo ganglion cell recordings reveal an unusual spike potential very similar to cardiac muscle. Physiologic parameters indicate that nerve degeneration is Wallerian in nature. A mild myopathy appears to be associated with Me-Hg. Pathologic lesions become obvious before physiologic alterations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In an

applied sense, fundamental knowledge of Me-Hg neurotoxicity may lead to improved diagnostic and therapeutic approaches to Me-Hg poisoning. In a basic sense, the interesting and unusual effects of Me-Hg on neuron membrane dynamics and structure may lead to a fundamental breakthrough in neuronal membrane excitability theory.

PROPOSED COURSE: Within two months data will be complete and ready for publication.

PUBLICATIONS

Somjen, G., and Herman, S.: Membrane potentials of spinal ganglion cells from methylmercury poisoned rats. Federation Proceedings. April 1972. (Abstract).

Somjen, G., and Herman, S.: Organic mercury poisoning: Electrophysiology of degenerating sensory ganglion cells. Science. (In Press).

Serial No.: NIEHS-PP-009
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Rat Kidney in Methylmercury Poisoning--Acute and Chronic Changes"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Ronald Klein, M.D.

OTHER INVESTIGATORS: Bill Bullock, D.V.M., Consultant, Bowman-Gray School of Medicine, Winston-Salem, North Carolina; and Sheldon Herman, M.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The object of the experiments was to describe the pathological changes in the kidneys of methylmercury treated rats.

METHODS EMPLOYED: Rats were treated with 2 and 10 mg/kg body weight methylmercury hydroxide according to two different dosage schedules: Acute - 5 daily doses of 10 mg Hg/kg body weight; Subacute - 15 doses of 2 mg Hg/kg body weight on a Monday, Wednesday, Friday schedule over a 5 week period. Animals were sacrificed at 5 days and 44 days after first dose by perfusion with 4% glutaraldehyde, and tissues prepared for light and electron microscopy using routine methods.

MAJOR FINDINGS: Acute histological changes in treated rat kidneys included tubular epithelial cell changes pregressing from mild focal degenerative change to widespread areas of necorsis and appearance of foci of regeneration at day 15. Lesions were found in the outer stripe of the outer medulla and affected both the distal and proximal tubules.

Chronic histological changes revealed an ongoing process of tubular epithelial cell hydropic degeneration and necrosis, regeneration and areas of interstitial nephritis which were predominantly distributed in the medullary rays in the midcortex. Glomeruli, papilla and blood vessels were normal.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nephrotoxic effects of inorganic mercurial compounds have been described in detail; in contrast, little is known about the nephrotoxic effects of alkyl-mercurials on the kidney. This study is part of a larger ongoing project to describe in detail the functional biochemical changes and correlate them with the ultrastructural changes in methylmercury treated rat.

PROPOSED COURSE: To continue the collection of clinical laboratory data in chronically treated rats and determine the effects of various compounds on renal lesion in methylmercury treated rat. Distribution of labeled methyl-mercury^{203} in different parts of the kidney is to be studied micro-radioauto-graphyly.

Serial No.: NIEHS-PP-011
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effects of Methylmercury Via the Milk in Nursing Neonate Rats"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Ronald Klein, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The object of this experiment is to determine the effect of methylmercury hydroxide on nursing neonates.

METHODS EMPLOYED: Pregnant female rats received 17 doses of 2 mg/kg methylmercury hydroxide on days 2-20 during their pregnancy. Neonates from each litter were sacrificed at weekly intervals over a 4 week period, and body and organ weights, protein, RNA and total mercury levels were determined. Tissues were prepared for routine histology.

MAJOR FINDINGS: No differences in litter size were found. Preliminary data suggest small differences in organ/body weight ratios in liver and kidney. Tissue distribution of Hg was as follows: Blood>Kidney>Liver>Brain from week 1-4. No changes were seen by light microscopy.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the developmental effect of low levels of methylmercury on rat neonates is important. Little is known about the effects exposure to low levels of alkylmercurials has in man at critical periods of growth and development.

PROPOSED COURSE: The future course of this project will depend on completed results.

Serial No.: NIEHS-PP-012
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effect of Glutamate Therapy in Methylmercury Poisoning in Adult Male Rats"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Ronald Klein, M.D.

OTHER INVESTIGATORS: P. E. Brubaker, Ph.D., Sheldon Herman, M.D., and G. W. Lucier, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: A protective effect of sodium glutamate in $HgCl_2$ poisoning in rats has been described. It is the object of this study to see if a similar protective effect of glutamate is found in methylmercury poisoned rats.

METHODS EMPLOYED: Rats were given a loading dose of sodium glutamate 2 and 0.4 kg orally X 2 days, followed by 7 daily doses of 10 mg methylmercury/kg. During the treatment with methylmercury hydroxide, and for eight days after, the animals continued to be treated orally every day with sodium glutamate. Animals were sacrificed on day 15, and mercury levels were determined in blood, brain and kidneys at that time.

MAJOR FINDINGS: All rats developed the clinical signs of methylmercury poisoning previously described (weight loss, ataxia and crossing). The glutamate rats were more severely affected earlier than the rats receiving water. Brain/blood ratios of total mercury in glutamate treated rats was higher than in water treated rats.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There are presently no therapeutic protection agents for methylmercury poisoning. Glumamate, a common additive to foods, had been previously found to protect rats treated with $HgCl_2$. The opposite results were found in methylmercury intoxication.

PROPOSED COURSE: A metabolic study on mercury distribution in the different groups at different times using larger groups of animals is planned.

Serial No.: NIEHS-PP-013
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Pathology of Acute Methylmercury Intoxication in Guinea Pigs"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Ronald Klein, M.D.

OTHER INVESTIGATOR: Stephen Falk, M.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Object was to identify the clinical, biohemical and gross effects and the light microscopic pathology resulting from administration of methylmercury hydroxide in guinea pigs.

METHODS EMPLOYED: Guinea pigs weighing 150 g were treated subcutaneously with daily doses of methylmercury hydroxide, 2 mg Hg/kg body weight, for 6 weeks. Animals were sacrificed at 1, 2, 4, 6 and 7 weeks after the first dose.

MAJOR FINDINGS: The only significant clinical change was marked loss in weight beginning at about the fourth week, along with decreased movement. Light microscopic changes involved almost exclusively the cerebellum,where a decreased number of internal granular cells and pyknoti nuclei were seen. Preliminary data show a slow but gradual uptake of mercury into the tissues. The total mercury tissue concentrations at day 48 after the first dose was: Kidney:Liver:Brain:Whole Blood.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is designed to compare and contrast species differences (rats, mouse, guinea pig) at low doses of methylmercury compounds. The toxic effect of long-term, low-level exposure in man as well as experimental animals is poorly understood.

PROPOSED COURSE: The plans are to continue low-dose long-term treatment of guinea pigs, monitoring mercury distribution and biochemical as well as light and electron microscopic changes.

Serial No.: NIEHS-PP-014
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "A Comparison of Conditioning in Rats Treated in utero with Methylmercury and Non-Treated Rats"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Ronald Klein, M.D., and Regenia Monday

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.2
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To determine if any variations occur between methylmercury treated and non-methylmercury treated rats with regard to their conditioning ability.

METHODS EMPLOYED: (1) Food reinforced behavior conditioning via the Skinner Box Test. (2) Experimental subjects--male rats that were newborns of experimental mothers (4 mothers treated with 2 mg/kg methylmercury hydroxide for 15 doses, 4 mothers treated with 0.1 ml H_2O for 15 doses). (3) Experimental environment included constant noise and light, regularly controlled feedings, free access to water and cages enclosed in a well ventilated hood.

MAJOR FINDINGS: Rats treated in utero with methylmercury: (1) required more training sessions and time to become conditioned to the extent of the control rats; (2) required longer periods of time after being placed in the Skinner Box before they pressed the lever for the first time as compared with controls; (3) more readily adjusted to extinction than did the controls; and (4) began reconditioning themselves after extinction more slowly than did the control rats.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In utero exposure to high doses of methylmercury would appear to have varied effects on the conditioning processes of rats, such as enhancing extinction adjustment and inhibiting reconditioning after extinction and normal conditioning as compared with non-methylmercury treated rats.

PROPOSED COURSE: Future plans include evaluation of differences in activity, brain weight, body weight, brain/body weight ratio and protein concentrations in conditioned and non-conditioned, treated and control rats.

Serial No.: NIEHS-PP-015
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Uptake of Methylmercury Hydoxide (Me-Hg) by Rat Nervous System"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Sheldon Herman, M.D., and Ronald Klein, M.D.

OTHER INVESTIGATOR: P. E. Brubaker, Ph.D.

COOPERATING UNITS: George Somjen, Ph.D., M.D., Consultant, Duke University, Department of Pharmacology and Physiology; Martin Krigman, M.D., Consultant, University of North Carolina at Chapel Hill, Department of Pathology; J. Goodrich, M.D., W. Briner, Ph.D., Duke University, Division of Nuclear Medicine.

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: Localization and quantitation of Me-Hg uptake in peripheral and central nervous system of the rat.

MAJOR FINDINGS: Uptake of Me-Hg^{203} by neural tissue was assayed by scintillation well spectrometry. Over a 4 week period Me-Hg accumulated principally in cerebral cortex, cerebellum and spinal ganglia. Uptake by nerves, roots and cord was significantly lower. Uptake by other organs (liver, fat, muscle) was also measured. Autoradiography further localized Me-Hg in spinal ganglion neuron soma in contrast to poor uptake by anterior horn cells.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies provide an explanation for the highly selective neurotoxicity of alkylmercurials. Distribution of pathology (i.e. spinal ganglion, cerebellum, cortex) correlates well with distribution of Me-Hg.

PROPOSED COURSE: Project complete

PUBLICATIONS

Herman, S., Somjen, G., Klein, R., Goodrich, J., Briner, W., Brubaker, P.: Uptake of Me-Hg^{203} by rat nervous system. Accepted for International Congress of Pharmacology, July 1972 (Abstract).

Krigman, M., Herman, S., Somjen, G., and Klein, R.: Pathology as related to uptake of Me-Hg^{203} in rat nervous system. J. of Exp. Neuropathology and Neurology. (In Press).

Herman, S., Krigman, M., Somjen, G., Klein, R., Talley, F., Brubaker, P., Briner, W., and Goodrich, J.: Methylmercury induced primary sensory neuropathy in the rat: Role of spinal ganglion. Science. April 1972. (In Press).

Serial No.: NIEHS-PP-016
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Subacute Neuropathology of Me-Hg in the Rat"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Sheldon Herman, M.D., and Ronald Klein, M.D.

OTHER INVESTIGATORS: Martin Krigman, M.D., Consultant, University of North Carolina at Chapel Hill, Department of Pathology, and Fred A. Talley

COOPERATING UNITS: None

MAN YEARS

Total:	0.7
Professional:	0.6
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: A general study of light and electron microscopy of Me-Hg neuropathology in the rat, with principal emphasis on peripheral nervous system (PNS).

METHODS EMPLOYED: Light and electron microscopy were used.

MAJOR FINDINGS: This study, begun in 1971, is completed. A model of subacute Me-Hg poisoning was developed and carefully described on the clinical and pathological levels. Rats develop PNS disease before central nervous system lesions. The peripheral neuropathy is sensory in distribution, begins in spinal ganglion neuron cell bodies, produces Wallerian-like degeneration in nerve fibers and precedes clinical evidence of disease by 1-2 weeks. Electron microscopy has revealed a typical pattern of organo-mercurial ultrastructural lesions in ganglion cells.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study provides the experimental model for further studies of Me-Hg neurotoxicity. It presents the first detailed electron microscopic study of Me-Hg poisoned ganglion cells, and thus firmly establishes the direct toxicity of Me-Hg on these cells.

PROPOSED COURSE: Project complete.

PUBLICATIONS

Krigman, M., Herman, S., Somjen, G. and Klein, R.: Pathology as related to uptake of Mr-Hg^{203} in rat nervous system. J. of Exp. Neuropathology and Neurology. (In Press).

Herman, S., Krigman, M., Somjen, G., Klein, R., Talley, F., Brubaker, P., Briner, W. and Goodrich, J.: Methylmercury induced primary sensory neuropathy in the rat: Role of spinal ganglion. Science. April 1972. (In Press).

Serial No: NIEHS-PP-017
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism, Function and Pathology of Methylmercury Hydroxide (Me-Hg) Poisoned Spinal Ganglion Neurons of the Rat In Vivo"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Sheldon Herman, M.D., and Ronald Klein, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: G. Somjen, M.D., Ph.D., Consultant, Duke University, Department of Pharmacology and Physiology, and M. Krigman, M.D., University of North Carolina at Chapel Hill, Department of Pathology

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Precise identification of the relationship of protein synthesis alterations in individual neurons of animals poisoned in vivo by Me-Hg administered parenterally over 2-5 weeks and of early and late membrane excitability and structural alterations.

METHODS EMPLOYED: Autoradiography and electron microscopy will be used.

MAJOR FINDINGS: Thus far an autoradiographic technique has been developed whereby neurons are labeled by L-Lysine ½ hour after IV injection, and reveal adequate grain density 3 days after emulsion exposure.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding of the pathophysiology of Me-Hg induced neuronal degeneration should be further advanced by this study.

PROPOSED COURSE: Following characterization of amino-acid incorporation patterns in poisoned ganglia, a second series of rats will be dosed with Me-Hg. The ganglion cells will be penetrated via intracellular electrodes, conduction properties measured, and individual cells marked with dye. After electrical

recordings, the animal will receive L-lysine IV, and ganglia stained with dye will be evaluated by autoradiography and electron microscopy.

Serial No.: NIEHS-PP-018
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Reports
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Heavy Metals on Hepatic Microsomal Enzymes"

PREVIOUS SERIAL NUMBER: NEIHS-CB-018

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: O. S. McDaniel, R. Klein, M.D., P. Brubaker, Ph.D., and H. B. Matthews, Ph.D.

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	1.1
Professional:	1.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The health hazards of organic mercury in our environment are becoming increasingly evident. However, most attention has been focused on neurological effects, with essentially no studies conducted on in vivo effects of the hepatic drug metabolizing enzymes. Our investigations were initiated to: (1) determine the effects of methylmercury hydroxide (Me-Hg) and other organic mercurials on microsomal enzyme systems (UDP glucuronyl-transferases and mixed function oxidases); (2) evaluate effects of organic mercurials on the metabolism of the carbamate pesticides, which require rapid hydroxylation and conjugation to be detoxified and excreted; (3) determine the mechanism by which organic mercurials decrease cytochrome P-450 and cytochrome b_5 content; (4) determine effects resulting from the interaction of inducing agents (chlordane, dieldrin, phenobarbital, and piperonyl butoxide) and organic mercurials on the drug-metabolizing enzymes; and (5) determine if the effects of methylmercury hydroxide (Me-Hg) on microsomal enzymes are consistent for a variety of species and for both males and females.

METHODS EMPLOYED: Livers from male and female rats, mice and guinea pigs were used for preparation of microsomes and for all other studies. Other relevant methods are described in the Major Findings section.

MAJOR FINDINGS: Effects on microsomal enzymes. The microsomal cytochromes,

P-450 and b_5, were markedly reduced following subcutaneous administration of Me-Hg at doses of 2.5 mg/kg/day for two days. The reduction was approximately 50% whether animals received 2.5 mg/kg/day or 10 mg/kg/day for 2 days. Doses of less than 2 mg/kg resulted in only a slight decrease of the microsomal cytochromes. The observed effects of Me-Hg on the rate of aminopyrine demethylation and aniline hydroxylation were identical to those observed for cytochrome P-450. Decreased levels of mixed function oxidase activity can therefore be explained by a decrease in hepatic cytochrome P-450 levels. At all dosage levels used, the animals exhibited no neurological symptoms, no weight loss and no liver histopathology as determined by light microscopy. Reduction of aminopyrine demethylase was maximal 24 hours after a single treatment of Me-Hg. The effect gradually diminished, so that by 1 week after treatment there was no effect on microsomal enzyme activity. The depression of cytochrome P-450 and associated enzymes was accompanied by a reduction of the smooth endoplasmic reticulum fraction (SER). The rough endoplasmic reticulum (RER) was markedly proliferated by Me-Hg treatment but this was not associated with an increase in mixed function oxidase activity. The observed biochemical effects on the submicrosomal fractions were verified by electron microscopy, which also demonstrated proliferation of RER at the expense of the SER. The large increase in RER was accompanied by a corresponding large increase in polyribosomes and monoribosomes while total RNA levels were unchanged by Me-Hg pretreatment. Type I and Type II substrate-binding spectra were both decreased 2-fold by Me-Hg pretreatments and otherwise were affected in the same manner as cytochrome P-450. Me-Hg pretreatments (5 mg/kg/day for 2 days) of male rats, mice and guinea pigs revealed that decreased mixed function oxidase activity was greatest in rats followed by mice, and guinea pigs were least affected. Hepatic cytochrome P-450 content in male rats was decreased by 50% compared to a 35% decrease in females, although cytochrome b_5 was decreased by 30% in both sexes. Enzyme reactions not mediated by cytochrome P-450, such as UDP glucuronyltransferase and NADPH cytochrome c reductase, were not affected at dosage levels that markedly decreased mixed function oxidase activity.

Increased degradation of CO-binding particles by Me-Hg. Incorporation of ^{3}H- δ-aminolevulinic acid (^{3}H-ALA) into hemoprotein of hepatic CO-binding particles (cytochrome P-450) is more rapid in Me-Hg treated than control rats, although maximum incorporation is similar in both cases. By following the fate of ^{3}H-ALA, it has been determined that cytochrome P-450 turnover occurs in two phases, a fast-phase component and a slow-phase component. Treatment with Me-Hg stimulates the synthesis of fast-phase hemoprotein and decreases synthesis of the slow-phase component. The half-life of the fast-phase hemoprotein is 5.5 hrs. in treated animals compared to 10 hrs. in controls, whereas the slow-phase half-lives are approximately 65 hrs. in both sets of animals. These data suggest that cytochrome P-450 content is reduced by increasing degradation of the fast-phase component and/or decreasing synthesis of the slow-phase component.

Effects of Me-Hg and chlordane on the metabolism of carbamate pesticides in rats. Methylmercury hydroxide pretreatments (10 mg/kg/day for 2 days) decreased hepatic microsomal cytochrome P-450 levels and mixed function oxidase activity over 50%, while chlordane pretreatments (20 mg/kg/day for 2 days) induced the oxidative enzymes nearly two-fold. Methylmercury hydroxide

pretreatments also decreased microsomal hydroxylation reactions of carbaryl and carbofuran to form 4-hydroxy carbaryl, N-hydroxymethyl carbaryl and 3-hydroxy carbofuran. Chlordane pretreatments increased hydroxylation rates at these positions and also increased the amount of carbamate-^{14}C equivalents detected in the water-extractable fraction. When chlordane and methylmercury hydroxide were administered simultaneously the effects balanced out so that carbamate metabolism using microsomes from this group was similar to controls. In whole animal metabolism studies, chlordane pretreatment increased the rate of urinary elimination of carbaryl and carbofuran equivalents. Methylmercury hydroxide, unexpectedly, also increased urinary excretion of both carbamate equivalents; mechanisms involved in the enhancement of urinary excretion were investigated. Chlordane and methylmercury hydroxide pretreatment did not qualitatively alter microsomal or urinary carbamate metabolites, although some quantiative differences were observed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Me-Hg exposures interfere with oxidative enzymes that function in the degradation of xenobiotics as well as in the normal metabolism of many endogenous compounds. These alterations in mixed function oxidase activity may interfere with the normal regulation of the steroid hormones. The carbamate pesticides, and many other environmental agents and drugs, are considered relatively safe because they are rapidly detoxified by hydroxylation reactions. However, if the normal rate of hydroxylation is inhibited, these compounds may become considerably more toxic to animals. Decreased mixed function oxidase activity by Me-Hg may also have implications in the administration of drugs to patinets who have been exposed to methylmercury because many of the drugs are metabolized by the mixed function oxidases. In our experiments hexobarbital sleeping times (rats) were increased by 250% in animals treated with MeHg at dosage levels which caused no neurological symptoms.

PROPOSED COURSE: Our research on the effects of Me-Hg on rat liver microsomal enzymes will attempt to (1) determine whether methylmercury or a metabolite is the active species in causing increased degradation of CO-binding particles in vivo; (2) quantify the extent of demethylation of methylmercury to form inorganic mercury in liver, kidney and brain; (3) determine mechanism of demethylation and whether demethylation occurs before or after binding to the endoplasmic reticulum; (2) investigate the relationship between chemotherapeutic agents and the metabolism and transfer of methylmercury in liver, kidney and brain; and (5) develop model systems to isolate metabolic intermediates.

We also plan to initiate studies on the effects of inorganic and organic lead on liver and kidney microsomal and lysosomal enzymes.

PUBLICATIONS AND ABSTRACTS

Lucier, G., Klein, R., McDaniel, O., and Brubaker, P.: Effects of methylmercury hydroxide on rat liver microsomal enzymes. Chem. Biol. Interact. 4: 265, 1972.

Lucier, G.W.: Effects of methylmercury and chlordane on the metabolism of carbamate pesticides. Entomological Society of America Convention, Los Angeles, Dec. 1971.

Lucier, G.W. and McDaniel, O.S.: Decreased hepatic mixed function oxidase activity by methylmercury in mammals. Fifth International Congress of Pharmacology, San Francisco, July, 1972.

Lucier, G.W., Klein, R., Matthews, H.B., and McDaniel, O.S.: Increased degradation of rat liver CO-binding particles by methylmercury. Life Sciences. (In Press)

Lucier, G.W., McDaniel, O.S., Williams, C., and Klein, R.: Effects of methylmercury and chlordane on the metabolism of carbaryl and carbofuran in rats. Pesticide Biochem. Physiol. (Submitted)

Lucier, G.W., McDaniel, O.S., Matthews, H.B., Brubaker, P., and Klein, R.: Further studies on the effects of methylmercury hydroxide on microsomal enzymes. Chem.-Biol. Interact. (In Preparation)

Serial No.: NIEHS-PP-019
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of the Carbamate Pesticides in Liver, Kidney, Lung, Stomach, Intestines and Blood: Effects of Chlordane and Methylmercury"

PREVIOUS SERIAL NUMBER: NIEHS-CB-019

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATOR: O. S. McDaniel

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.3
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: Mammalian metabolism of carbaryl and carbofuran, two of the more widely used carbamate pesticides, has been evaluated almost entirely on the basis of urinary metabolites. However urinary metabolism studies are more an indices of excretion rates rather than metabolism. In our studies we plan to investigate the nature of metabolites present in stomach, intestine, blood, liver, kidney and lung as well as in the urine. These experiments will be designed to yield data concerning the following: (1) the rate of absorption of the carbamates through the stomach and intestine and whether the stomach and intestine degrade the carbamates before transport to liver and kidney; (2) levels of parent compounds and the nature of metabolites present in each organ at varying times after exposure to the carbamates; (3) length of time toxic (cholinesterase-inhibiting) metabolites remain in each organ; (4) *in vitro* metabolism of the carbamates using stomach, intestine, lung, liver and kidney tissues as the enzyme source; and (5) whether animals exposed to other environmental agents (chlordane and methylmercury) differ from control animals with respect to the above mentioned criteria.

METHODS EMPLOYED: Male rats are used in the metabolism studies. Chlordane is administered orally and methylmercury hydroxide subcutaneously. Radiolabeled carbaryl and carbofuran are used to evaluate metabolism and are administered orally and as aerosols. Metabolites are identified by

co-chromatography (thin-layer plates, DEAE-cellulose columns and florisil columns) and isotope dilution techniques.

MAJOR FINDINGS: The half-life of carbaryl-1-naphthol-^{14}C equivalents in the stomach and small intestines following oral administration is approximately 25 minutes. The half-life for carbofuran-ring-^{14}C equivalents is approximately 20 minutes. Radioactivity in the stomach and small intestine following oral administration of carbaryl-^{14}C consisted of 90% unmetabolized parent compound, and 5% conjugated metabolites; the remaining 5% included 1-naphthol, N-hydroxymethyl carbaryl and 4-hydroxy carbaryl. Most of the carbaryl-^{14}C equivalents in liver and kidney was in the form of conjugated metabolites. There were trace levels of unmetabolized parent compound and hydroxylated unconjugated metabolites. Rat liver microsomes and homogenates metabolize carbaryl primarily to form N-hydroxymethyl carbaryl, 4-hydroxy carbaryl, 5-hydroxy carbaryl and 1-naphthol in addition to several trace metabolites. The major microsomal metabolites of carbofuran were 3-hydroxy carbofuran and carbofuran phenol.

Pretreatment of rats with methylmercury decreased microsomal mixed function oxidase activity towards carbaryl and carbofuran by over 50%. Chlordane pretreatments had the opposite effect and induced oxidative activity towards both carbamates. Methylmercury and chlordane had no significant effect on the rate of absorption of the radiolabeled carbamates through the stomach and small intestine. Preliminary data indicate that rats pretreated with chlordane had a higher percentage of conjugated carbaryl metabolites in stomach, intestine, kidney and liver than controls.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: With the increasing use of the less persistent carbamate and organophosphate pesticides, it is necessary to have information concerning their rate of enzymatic degradation as modified by other environmental agents for evaluation of potential hazards. Inducers of oxidative and conjugative enzymes will generally protect animals against pesticide poisoning, whereas inhibitory agents should cause the animal to be more susceptible. Since the acute toxicity of carbamates occurs shortly after exposure (before urinary collections can easily be made), it is useful to determine the nature of metabolites present in various organs and blood before significant urinary elimination occurs. Mammalian exposures to the carbamates occurs primarily through the stomach and lungs. Therefore it is also important to know whether the carbamates are metabolized in stomach, intestine and lung before transport to the liver and kidneys and whether simultaneous exposures to other environmental agents alter the normal rate of absorption.

PROPOSED COURSE: Studies will continue to evaluate qualitatively and quantitatively the metabolism of carbaryl and carbofuran in a variety of mammalian tissues. Both *in vivo* and *in vitro* systems will be used.

Serial No.: NIEHS-PP-020
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Microsomal Rat Liver UDP Glucuronyltransferase: Purification and Alterations in Activity by Piperonyl Butoxide and Other Factors"

PREVIOUS SERIAL NUMBER: NIEHS-CB-020

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: O. S. McDaniel, H. B. Matthews, Ph.D., and P. E. Brubaker, Ph.D.

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To characterize optimal *in vitro* reaction conditions and to develop rapid and sensitive assay techniques for measuring activity of conjugative enzyme systems; (2) to solubilize and purify glucuronyltransferase in order to characterize the enzyme and determine its specificity and multiplicity and (3) to evaluate *in vitro* inhibition of conjugative enzymes by insecticide synergists on conjugative enzymes with special reference to enzyme induction and protein synthesis.

METHODS EMPLOYED: Livers from male and female rats (CD strain, 125-150 g) were used for preparation of microsomes and all other studies. Other relevant methods are described with the Major Findings.

MAJOR FINDINGS: Activation of rat liver microsomal UDP glucuronyltransferase by detergent or aging was not accompanied by a change in Km for 1-naphthol conjugation or UDPGA. Divalent cations (MG^{++}, Mn^{++}, and Co^{++}) at 10 mM stimulated glucuronic acid conjugation of 1-naphthol and p-nitrophenol in both unactivated and activated microsomes and in both smooth and rough-surfaced endoplasmic reticulum. However, Zn^{++} (10 mM) strongly inhibited UDP glucuronyltransferase. A total activation factor of 30-fold resulted when Triton X-100 and $MgCl_2$ were added to the incubation medium. The UDP

glucuronyltransferases conjugating 1-naphthol, testosterone and a dieldrin metabolite were inhibited *in vitro* by insecticide synergist chemicals. Piperonyl butoxide had I_{50} values of 7.3×10^{-6} M and 9.0×10^{-5} M for testosterone and 1-naphthol glucuronyltransferase, respectively. Pretreatment of male and female rats with piperonyl butoxide for three days resulted in a 2-fold induction of the UDP glucuronyltransferase conjugating 1-naphthol and p-nitrophenol. L-Naphthol and p-nitrophenol were shown to be glucuronidated by the same enzyme by analysis of the mixed substrate method, divalent cation requirements, activation by detergent or aging, submicrosomal distributions, levels of induction by piperonyl butoxide and gravitational studies. This work was greatly facilitated by the use of a new, rapid and sensitive radioassay for UDP glucuronyltransferase which, along with the above results, has been published.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: UDP glucuronyltransferases play an important role in the metabolism and excretion of numerous xenobiotics and endogenous compounds. Any disruption in the detoxication rate of foreign compounds or rate of metabolism and excretion of endogenous compounds might result in a serious health hazard. Insecticide synergists which are commonly used in household sprays and in conjunction with many other insecticide applications were believed to exert their primary action by serving as alternative substrates for the mixed function oxidase enzymes. However, evidence presented here indicates that the UDP glucuronyltransferases are involved as well. The radiolabeled assay procedure described here has applications for many other enzyme assays, such as the β-glucuronidases, sulfatases, esterases and sulfokinases. It is also important to determine whether one enzyme is responsible for all glucuronidations or whether several more specific enzymes are operative, i.e., one enzyme which operates in steroid metabolism and another in conjugation of phenolic foreign compounds.

PROPOSED COURSE: Future studies will concentrate on the purification of glucuronytransferase in order to more adequately characterize the enzyme, gain insight into its biosynthesis and evaluate enzyme inhibition by some insecticide synergists.

PUBLICATIONS

Lucier, G.W., McDaniel, O.S., and Matthews, H.B.: Rat liver microsomal glucuronyltransferase: Effects of insecticide synergists and other factors on enzyme activity. Arch. Biochem. Biophys. 145: 520, 1971.

Lucier, G.W. and Brubaker, P.E.: Induction of microsomal enzymes, protein synthesis and polyribosomes by organochlorine pesticides and insecticide synergists. (In Preparation)

Serial No.: NIEHS-PP-021
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Alterations in Rat Liver Microsomal and Lysosomal β-Glucuronidase by Compounds Which Induce Hepatic Drug-Metabolizing Enzymes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: O. S. McDaniel and P. E. Brubaker, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.6
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: There are numerous reports on the properties of lysosomal β-glucuronidase in a variety of tissues. However β-glucuronidase is also a structural component of the endoplasmic reticulum. Because agents such as the organochlorine pesticides induce several microsomal enzymes (mixed function oxidases and UDP glucuronyltransferases), it is of interest to evaluate the effects these agents have on microsomal β-glucuronidase. Experiments will be designed to determine whether lysosomal and microsomal β-glucuronidase are the same enzyme. We also plan to determine if there is a relationship between alterations in microsomal β-glucuronidase and induction of the drug-metabolizing enzymes. The enzyme will be purified to determine if exposures to organochlorine pesticides change the properties of microsomal β-glucuronidase.

METHODS EMPLOYED: Male and female rats are used as the enzyme source, and chlordane, dieldrin, benzpyrene and piperonyl butoxide are used as the inducing agents. Microsomal β-glucuronidase is solubilized by ribonuclease and ultrasound. Purification is accomplished by ammonium, sulphate fractionation, organic solvent fractionation, gel electrophoresis and migration in sucrose-density gradients.

MAJOR FINDINGS: Chlordane, dieldrin, piperonyl butoxide and benzpyrene, which induce the hepatic microsomal mixed function oxidases and UDP glucuronyltransferases, decreased activity of smooth and rough endoplasmic reticulum β-glucuronidase. The reduction occurred when either p-nitrophenyl β-D-glucuronide or phenolphthalein mono-β-glucuronide was used as the substrate. Chlordane or dieldrin pretreatment of rats for 3 days resulted in a 2.5-fold reduction in endoplasmic reticulum activity, while the reduction was less for piperonyl butoxide or benzpyrene. On the other hand, aminopyrine demethylase and UDP glucuronyltransferase were increased 2-fold by chlordane or dieldrin pretreatments. Decreases in microsomal glucuronidase activity might be directly or indirectly involved in the induction process because decreases in β-glucuronidase activity are quantitatively similar to increases in activity of the drug-metabolizing enzymes. Lysosomal β-glucuronidase also decreased following pretreatment of rats with inducing agents, but the reduction was less than that observed in the endoplasmic reticulum fractions. Analysis of pH optima, temperature optima, K_m values, heat denaturation data and effects of Triton X-100 on activities of various liver fractions suggests that β-glucuronidases from the endoplasmic reticulum fractions. Analysis of pH optima, temperature optima, K_m values, heat denaturation data and effects of Triton X-100 on activities of various liver fractions suggests that β-glucuronidases from the endoplasmic reticulum and lysosomes have similar properties. Preliminary studies, using enzyme purified 60-fold, revealed that incorporation of valine - ^{14}C into microsomal β- glucuronidase is slower in dieldrin-treated rats compared to controls. These studies also revealed that K_m values for phenolphthalein mono-β-glucuronide are higher in dieldrin-treated rats (4.1×10^{-4}M) than controls (1.3×10^{-4}M). Both total activity and specific activity of the purified enzyme were decreased nearly 4-fold by dieldrin pretreatments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The precise relationship between reductions in microsomal β-glucuronidase and induction of the drug-metabolizing enzymes remains unresolved. However, a possible explanation of the results is that the specific activity of endoplasmic reticulum β-glucuronidase would decrease if synthesis of endoplasmic reticulum protein is directed towards detoxifying enzymes in the induction process. If microsomal β-glucuronidase functions by hydrolysis of glycolipids and glycoproteins in the membrane structure causing turnover of the endoplasmic reticulum, then agents such as chlordane and dieldrin that reduce specific activity of β-glucuronidase would result in the proliferation process. Another possible explanation is that exposure to the organochlorine pesticides causes the synthesis of a microsomal β-glucuronidase having different properties than that found in control animals. Because glucuronidase activity in some tissues is markedly altered in many disease conditions, it is important to evaluate any changes in activity resulting from exposure to environmental agents. It is also improtant to determine the site of biosynthesis of this enzyme.

PROPOSED COURSE: Further experiments will be planned to obtain more information concerning the following: (1) the function of microsoma β-glucuronidase; (2) the relationship between decreased microsomal β-glucuronidase and induction of the drug-metabolizing enzymes; (3) whether pretreatment with organochlorine pesticides causes the synthesis of a

different microsomal β-glucuronidase; and (4) whether purified microsomal and lysosomal β-glucuronidase have the same properties.

PUBLICATIONS

Lucier, G.W. and McDaniel, O.S.: Alterations in rat liver microsomal and lysosomal β-glucuronidase by compounds which induce hepatic drug-metabolizing enzymes. Biochem. Biophys. Acta. 261: 168, 1972.

Serial No.: NIEHS-PP-022
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Methylmercury on Serum and Brain Cholinesterase Activity in the Rat"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: G. W. Lucier, Ph.D., and
F. Hastings, Ph.D. (U.S. Forestry Service)

OTHER INVESTIGATOR: R. Klein, M.D.

COOPERATING UNITS: U.S. Forestry Service, Research Triangle Park, N.C.

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Methylmercury pretreatment decreases rat liver microsomal mixed function oxidase activity in rats. These enzymes function in the detoxication of foreign compounds, such as the carbamate and organophosphate pesticides, which produce their toxic effect on mammals by inhibiting acetylcholinesterase activity. Therefore, information concerning the effect of methylmercury on cholinesterase activity is necessary to evaluate interactions between these pesticides and methylmercury.

METHODS EMPLOYED: Male and female rats were treated with methylmercury hydroxide for 2 days (5 mg/kg/day). Blood was drawn by heart puncture and brains removed following decapitation. Titrametric determinations of cholinesterase were accomplished using butyrylcholine as the substrate for serum cholinesterase and acetylcholine for brain cholinesterase.

MAJOR FINDINGS: Methylmercury pretreatment decreased female serum cholinesterase approximately 80%, whereas brain cholinesterase activity was not significantly changed. Neither brain nor serum cholinesterase activity was changed by Me-Hg pretreatment of males. Female serum cholinesterase levels were highly variable in control rats and were approximately four times more active than enzyme from control males. Serum cholinesterase levels from treated females were similar to control males. These studies also indicate

that methylmercury potentiates the toxicity of carbamate and organophosphate pesticides by decreasing cholinesterase levels as well as by inhibiting microsomal mixed function oxidases.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Brain cholinesterase (acetylcholinesterase) functions in synaptic nerve transmission. However the function of serum cholinesterase is unknown, although it is used to detect organophosphate and carbamate poisoning. Serum cholinesterase levels might reflect methylmercury intoxication in females, but not in males according to our studies with rats.

PROPOSED COURSE: Experiments are in progress to determine (1) the lowest dose of methylmercury that inhibits serum cholinesterase in females; (2) whether the high variability in serum-cholinesterase activity in females is related to the estrus cycle; and (3) whether testosterone administrations protect females against decreases in brain cholinesterase activity in methylmercury treated rats.

Serial No.: NIEHS-PP-023
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Environmental Agents on the Biochemistry and Biogenesis of Mitochondria"

PREVIOUS SERIAL NUMBERS: NIEHS-CB-01-10, NIEHS-CB-003

PRINCIPAL INVESTIGATOR: B. Dean Nelson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: (1) To study the interaction of toxic agents with mitochondrial membranes through analysis of oxidative phosphorylation and related enzyme reactions. (2) To elucidate the action of environmental agents on membrane formation during biogenesis of mitochondria.

MAJOR FINDINGS: Cyclodiene pesticides. Work is continuing to elucidate the mechanism by which these pesticides interfere with oxidative phosphorylation. Recent experiments show that the concentration of chlordane needed to inhibit respiration in submitochondrial particles (SMP) by 50% (I_{50}) is 20 to 40 times greater than that needed to inhibit respiration in intact, phosphorylating mitochondria. Only minor differences are observed in the I_{50} for chlordane for respiration in SMP when NADH, succinate or ascorbate+TMPD are used as substrates. These substrates assay 3, 2 and 1 phosphorylating sites respectively. Such experiments show that chlordane does not inhibit at a specific site in the electron transport chain, but rather acts on the reactions which transfer the respiratory chain-generated energy into the synthesis of ATP. Whether chlordane acts on the membrane to dissipate a proton (electrochemical) gradient, or whether it inhibits a specific enzyme involved in the energy transfer process cannot be decided at present. These two choices currently represent the two major views concerning the mechanism of energy conservation and transfer during oxidative phosphorylation. An understanding of the action of chlordane will, therefore, require resolution of the more basic problems of oxidative phosphorylation. Certain experiments using chlordane, however,

support the concept that a high energy chemical intermediate is stabilized by the pesticide. Respiration is stimulated by arsenate, according to the chemical hypothesis of oxidative phosphorylation, by replacing the phosphate of a hypothetical phosphorylated intermediate with an unstable arsenate bond. The observation that low concentrations of chlordane inhibit arsenate-induced respiration, whereas high concentrations of the pesticide reactivate respiration, suggests that in the former case chlordane acts to stabilize an intermediate in the reaction. Further experiments will be conducted on the feasibility of using chlordane as a tool for investigating the process of oxidative phosphorylation.

Methylmercury (Me). In intact, phosphorylating rat liver mitochondria Me inhibits both ADP-and dinitrophenol (DNP)-activated respiration, but has little effect on substrate respiration with glutamate, succinate or ascorbate+ TMPD as the substrates. The concentration of Me required for 50% inhibition (I_{50}) is in the order of 10-15 nmoles Me/mg protein, and appears to be related inversely to number of uncoupled sites involved, i.e., the concentration needed for 50% inhibition increases in the order glutamate, succinate and ascorbate+ TMPD. The observation that uncoupler-stimulated respiration is inhibited by Me suggests that the action of Me may be directly on the respiratory chain. However, studies on SMP eliminate this possibility because the concentration of the mercurial needed to inhibit respiration in SMP by 50% was 40 to 100 times greater than in phosphorylating mitochondria. These findings suggest that Me acts either by inhibiting the phosphorylation reactions or by inhibiting transport of anions (substrate, DNP or phosphate) across the membrane. This conclusion is supported by the observation that under contitions where the ADP:O ration and ADP-activated respiration are inhibited by Me, substrate respiration is neither inhibited nor accelerated as would be the case in uncoupled mitochondria.

In SMP, succinate oxidate is more sensitive to inhibition than NADH oxidase suggesting that Me acts directly on succinate dehydrogenase (SDH) under certain conditions. These findings agree with published results showing inhibited SDH activity in mitochondria isolated from rats pretreated with Me.

The inhibitory action of Me apparently involves binding of the mercurial to either a sulfhydryl or a dithiol group because cysteine completely reverses the inhibited state.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effect of environmental agents on mitochondrial biochemistry and cellular energy metabolism is an important consideration in environmental health today. The present studies indicate for the first time the possibility that certain chlorinated pesticides, whose toxic action heretofore were thought to be nonspecific, act rather specifically by interfering with energy transfer reactions in mitochondria. These findings point to the need for substantial work on alterations in cellular metabolism which result from inhibitory, yet nontoxic, concentrations of pesticides at the site of cellular control, i.e., the mitochondria.

PROPOSED COURSE: Studies will continue on the binding of toxic agents to mitochondria membranes and the resulting alterations in mitochondrial function

and morphology. The action of toxic agents on membrane formation will be investigated in vitro with submitochondrial particles. The work with microorganisms will be continued since these organisms afford a test system in which mitochondrial genesis can be controlled through manipulation of the growth media.

PUBLICATIONS

Nelson, B.D.: Action of the fungicides Captan and Folpet on rat liver mitochondria. Biochem. Pharmacology. 20: 737-748, 1971.

Nelson, B.D.: Induction of mitochondrial swelling by the fungicide Captan. Biochem. Pharmacology. 20: 749-758, 1971.

Nelson, B.D., Drake, R., and McDaniel, O.: Effects in vitro and in vivo of methylenedioxyphenyl compounds on oxidative phosphorylation in rat liver mitochondria. Biochem. Pharmacology. 20: 1139-1149, 1971.

Nelson, B.D., and Williams, C.: Action of Cyclodiene pesticides on oxidative metabolism in the yeast Saccharomyces cerevisiae. Agricult. and Food Chemistry. 19: 339-341, 1971.

Serial No.: NIEHS-PP-024
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Role of Coenzyme Q and Cytochrome b in Oxidative Phosphorylation"

PREVIOUS SERIAL NUMBER: NIEHS CB-004 and 005

PRINCIPAL INVESTIGATOR: B. Dean Nelson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: To study the role of UQ and cytochrome b in electron transport and in energy conservation reactions in mitochrondria.

MAJOR FINDINGS: In phosphorylating submitochondrial beef heart particles, electron transport can be driven up a normally thermodynamically unfavorable gradient, if energy is supplied externally. Thus, electrons from ascorbmate+ N,N,N',N'-tetramethylphenylenediamine (TMPD) enter the electron transport chain at cytochrome c, reducing all of the cytochromes aa_3 and cytochromes c_1 and a small portion of cytochrome b. Addition of ATP results in further reduction of cytochrome b because, under these conditions, the thermodynamic equilibrium is shifted and electrons can move across the energy barrier (site II phosphorylation) which exists between cytochrome c_1 and cytochrome b. Complete reversal of the electron transport chain is accomplished upon addition of NAD^+ in the presence of ATP as shown by the formation of reduced NAD^+. It has always been assumed that addition of NAD^+ under these conditions would result in oxidation of cytochrome b because its reduction by cytochrome c_1 involves transport of electrons across an energy gap, and should be rate limiting. Our experiments, however, show that addition of NAD^+ results in a further reduction, not an oxidation, of cytochrome b. This NAD-inducing reduction of cytochrome b is energy-dependent and occurs only in the presence of ATP. In addition, the cytochrome b reduced by addition of NAD^+ is different from that reduced upon addition of ATP. The former has a spectral peak for the alpha band at 562 nm whereas the spectral peak of the latter is at 565 nm.

Further studies show that (1) inclusion of a NAD^+ regenerating system, such as ethanol and alcohol dehydrogenase, prevents and reverses the NAD-induced reduction of cytochrome b; and (2) catalytic amounts of NAD^+ (which are rate limiting in the reaction) result in the same steady state reduction of cytochrome b irrespective of the initial rates. These results show that NADH is required for reduction of the cytochrome b absorbing at 562 nm, and indicates that two separate respiratory chains are involved. This was further substantiated by experiments showing that addition of NAD^+ results in the additional reduction of 50% of the total flavoprotein and Coenzyme Q. The results, therefore, demonstrate that beef heart particles are composed of two separate pools of Coenzyme Q and flaboprotein and which are connected to two spectroscopically distinct cytochrome b moieties. Tentatively we have concluded that the two chains converge at cytochrome c_1 although additional experiments will be needed to confirm this. Furthermore, the relationship of these chains and the two cytochromes to energy conservation at site II phosphorylation remains to be elucidated. A rule for two cytochrome b (absorbing at 562 nm and 565 nm) species in energy conservation at site II has been suggested by Chance and coworkers.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Previous studies have shown that many environmental agents are potent inhibitors of electron transport and/or oxidative phosphorylation. Only in a few cases, where unusual specifity obtains, is the specific site of interference known. An impediment to full understanding of the mode of operation of environmental agents in these processes is a basic lack of information about details of the electron transport system itself. These studies are designed to clarify the components and mechanisms of electron transport and, subsequently, to facilitate understanding of interference mechanisms of specific environmental agents.

PROPOSED COURSE: Work will continue to elucidate the interaction of Coenzyme Q and cytochrome b, as well as their respective roles in electron transport and energy conservation.

PUBLICATIONS

Nelson, B.D., Norling, B., Persson, B., and Ernster, L.: Effects of thenoyltrifluoroacetone on the interaction of succinate dehydrogenase and cytochrome b in ubiquinone-depleted submitochrondiral particles. B.B.R.C. 44: 1312-1320, 1970.

Nelson, B.D., Norling, B., Persson, B., and Ernster, L.: Effects of certain non-chelators and antibiotics on the interactions of succinate dehydrogenase and cytochrome b in uniquinone-depleted submitochrondrial particles. B.B.R.C. 44: 1321-1329, 1971.

Nelson, B.D., Norlong, B., Persson, B., and Erster, L.: Regulation of the interaction of succinate dehydrogenase and cytochrome b by ubiquinone. (Abstr.) Proc. Amer. Chem. Soc. Meeting. Symposium in Thermodynamic basis for energy coupling mechanisms. Washington, D.C., Sept. 1971.

Nelson, B.D., Norling, B., Persson, B., and Ernster, L.: Regulation of the interaction of succinate dehydrogenase and cytochrome b. International

Symposium on the Biochemistry and Biophysics of Mitochondrial Membranes. Bressanone, Italy, Academic Press, 1971. (In Press).

Nelson, B.D., Norling, B., Persson, B., and Ernster, L.: Influence of ubiquinone on the rate of antimycin binding to submitochrondrial particles. BBA. (In Press).

Serial No.: NIEHS-PP-025
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Heavy Metal Intoxication"

PREVIOUS PROJECT NUMBER: None

PRINCIPAL INVESTIGATOR: R. A. Goyer, M.D., Visiting Scientist

OTHER INVESTIGATORS: Drs. K. R. Mahaffey and B. C. Rhyne, University of North Carolina at Chapel Hill

COOPERATING UNITS: Department of Pathology, University of North Carolina at Chapel Hill

MAN YEARS

Total:	1.3
Professional:	0.3
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are (1) to identify cellular effects of heavy metals, particularly adaptation of detoxifying mechanisms and metabolic factors which influence pathological effects; and (2) to study the influence of orally ingested NTA (nitrilotriacetic acid), a detergent builder, on metabolism of trace metals in vivo in rats. Particular attention is given to the the hyperglycemic effects of NTA and a characteristic vacuolar lesion occurring in proximal renal tubular cells.

METHODS EMPLOYED: (1) Effects of solutions containing trace amounts (10^{-3} to 10^{-5} molar) of lead, bismuth and arsenic on growth rates and ultrastructure of in vitro cultures of Hela cells, Green Monkey kidney cells and "L" cells are studied. (2) The toxicity of NTA, a metal chelator, has been studied in rats. Effects of particular interest are influence on blood sugar levels, influence of chromium and zinc supplementation on NTA effects on carbohydrate metabolism and ultrastructural characteristics of vacuoles occurring in proximal renal tubular lining cells of NTA treated rats.

MAJOR FINDINGS: Lead concentrations added to culture media or fetal calf serum in the order of 10^{-3} molar inhibit cell growth in tissue culture. The characteristic ultrastructural changes (mitochondrial changes and nuclear inclusion bodies) observed in renal tubular lining cells of rats fed lead in vivo do not develop in lead treated tissue cultures. Tissue culture cells exposed to lead

accumulate amorphorous precipitated material presumed to be lead within the cytoplasm. The interpretation of this observation is that lead is not transported (in the form given) into tissue culture cells and is not metabolized in the way that lead must be to be transported through a renal tubular lining cell *in vivo*. Similar results are found with *in vitro* feeding of bismuth.

Arsenic, in the form of a solution of disodium arsenate (20 ppm), administered to Hela cell cultures inhibits cell growth and produces a variety of ultrastrucutral effects on mitochrondria which resemble effects observed in kidney cells when disodium arsenate is administered to rats *in vivo*. These include formation of paracrystalloid lattices in mitochondria and an amorphous flocculent density within the mitochondria matrix, bizarre shapes and enlargement of mitochondria.

Trisodium NTA, at either 0.01%, 0.10%, or 1% (w/v), was given to rats in drinking water with or without 200 µg Pb/ml. At the 1% NTA level, rats became ill and were autopsied after four weeks of the experiment. These rats had a vacuolar tubular nephropathy, hyperglycemia and a marked glycosuria. Rats given 0.01% and 0.10% NTA with or without Pb displayed no histologic changes in kidney, liver, brain or pancreas but had elevated blood glucose levels. NTA alone lowered Pb content of kidneys, whereas NTA administered with Pb did not enhance Pb deposition of kidney. The hyperglycemic effect of NTA may be related to trace metal chelation.

PUBLICATIONS

Goyer, R.A.: Research needs regarding health effects of heavy metals in man. In *Proceedings of Water Resources Workshop*. Raleigh, North Carolina, August 17, 1971.

Goyer, R.A., and Rhyne, B.C.: Pathologic effects of lead. In Richter, G., and Epstein, M.A. (Eds): *International Reviews of Experimental Pathology*. Academic Press, Vol. 11, 1972. (In Press).

Goyer, R.A., and Rhyne, B.C.: Toxic changes in mitochondrial membranes and mitochondrial function. In Trump, B., and Arstila, A. (Eds): *Pathologic Aspects of Membranes*. Academic Press. (In Press).

Mahaffey, K.R., and Goyer, R.A.: Metabolic and renal effects of oral trisodium nitrilotriacetate (NTA) ingestion in control and lead-fed rats. *Arch. Environ. Health*. (In Press).

Serial No.: NIEHS-PP-026
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Placental Passage of Diquat and Paraquat and Concentrations in Fetal and Other Tissues: Relative Toxicities to Fetus, Neonate and Young Rat"

PREVIOUS SERIAL NUMBER: NIEHS-CB-026

PRINCIPAL INVESTIGATORS: Herbert S. Posner, Ph.D., B. Clair Eliason, M.D., and Charles W. Sharp, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Animal Science and Technology Branch and Biometry Branch

MAN YEARS

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: There are two objectives: (1) to compare the placental passage of two quaternary ammonium herbicides and their relative accumulations in various tissues, and (2) to compare the toxicities that occur in the fetus and neonate after maternal administration and in 20 day old rats on direct administration of these young rats.

METHODS EMPLOYED: Both ^{14}C-diquat and ^{14}C-paraquat were administered to the maternal rat. ^{14}C-Paraquat was also administered maternally. *In utero* deaths and resorptions were counted. Newborn and 20 day old rats were observed for reduction of weight gain, other signs of toxicity and early death.

MAJOR FINDINGS: Paraquat accumulated over 24 hours in the fetal lung, while the concentrations remained almost constant in fetal brain and liver and in the amniotic fluid and decreased in fetal kidney over this time. Diquat concentrations, while they achieved initially higher levels than the paraquat, declined over 24 hours. Paraquat concentration increased in neonatal lung for 2 days after birth and then declined. It was assayed in the neonatal lung, liver, kidney and muscle for up to nine days after birth. Diquat was more toxic than paraquat to the fetus, but similar and lower incidences of maternal deaths occurred. Neonates that came from severely affected, paraquat-

treated mothers appeared normal and gained weight normally when they were cross-fostered on non-treated mothers. They developed poorly with their own mothers. Twenty-day-old rats administered paraquat orally could be assigned, as in the case of adult rats, into groups that were "minimally affected" or that were "severely affected", that is continuously lost weight and died.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Quaternary ammonium compounds generally cross the placenta to a limited extent. However, transient effects have been observed in human neonates when drugs containing the quaternary ammonium group have been used for therapy. Paraquat has caused over 20 human deaths. It accumulates in the lung and causes death secondary to pulmonary pathology. There has been little investigation of the potential for similar or alternate effects on fetuses and neonates. The study investigates this potential. The results are to be compared to results obtained after administration of environmental chemicals of other structural types.

PROPOSED COURSE: ^{14}C-Paraquat will be administered to pregnant guinea pigs to determine fetal tissue uptake and release for comparison with that found in the rat. Several stages of gestation will be utilized. Experiments will also be performed in the guinea pig using the *in situ* perfused placental preparation after the 50th day of gestation. Several compounds will be tested for their ability to modify the amount of ^{14}C-paraquat that crosses either to or from the fetus.

Serial No: NIEHS-PP-027
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Modification of Placental Passage of Dieldrin"

PREVIOUS SERIAL NUMBER: NIEHS-CB-027

PRINCIPAL INVESTIGATORS: Herbert S. Posner, Ph.D., and B. Clair Eliason, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Animal Science and Technology Branch

MAN YEARS

Total:	0.8
Professional:	0.4
Others:	0.4

PROJECT DESCRIPTION

OBJECTIVES Conditions that alter the amount of a neutral, chlorinated hydrocarbon pesticide (dieldrin in this case) that crosses the placenta, either in the direction of the fetus or back to the mother, are being investigated. The potential for such interaction is being sought with drugs in common obstetrical use, with additional environmental agents to which the pregnant female might be exposed and following other forms of maternal exposure or variation of internal milieu. Knowledge of the mechanisms of the responses could permit rational use of the information obtained.

METHODS EMPLOYED: The guinea pig placenta was prepared for prefusion in situ. ^{14}C-Dieldrin was then administered maternally, and the maternal and fetal sides of the placenta were monitored for several parameters. Diluted human serum and purified human serum proteins were perfused on the fetal side of the placenta to determine their effects on transport of radioactivity.

MAJOR FINDINGS: Passage of ^{14}C-dieldrin from the mother to the fetus was highly dependent both on the concentration of total serum protein and on the type of protein perfused on the fetal side of the placenta. Response to changes in concentration of total protein was rapid. Alpha-globulins were most effective, beta-globulins, albumin and fibrinogen considerably less effective and gamma-globulins least effective in their ability to attract dieldrin from mother to fetus.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Placental passage depends upon a variety of factors such as the maternal and fetal blood concentrations of test substance, binding strengths to blood and tissue components, blood flow in the placenta and length of time that the substance remains in the maternal organism. In the present extension of our work, we determined some factors that tend to increase the concentration of dieldrin in the fetus, against which one would have to work either to reduce passage to the fetus or bring about depletion from fetal stores.

PROPOSED COURSE: Additional compounds will be administered to the maternal rat to determine if they can affect the transport of ^{14}C-dieldrin either to or from the fetus. Tests will be made at several stages of gestation. Compounds effective in the rat will be tested in the guinea pig and in other species. Some tests will be made using the *in situ* perfused guinea pig placenta. When compounds are found to affect transport of ^{14}C-dieldrin, attempts will be made to learn the mechanism of the response. Differences or similarities will be sought for effects in fetal and maternal tissues occurring in the same pregnant animal.

PUBLICATIONS

Eliason, B.C., and Posner, H.S.: Reduced passage of carbon-14-dieldrin to the fetal rat by phenobarbital but not by eight other drugs or dieldrin. Amer. J. Obstet. Gynecol. 110: 943-947, 1971.

Eliason, B.C., and Posner, H.S.: Placental passage of ^{14}C-dieldrin altered by gestational age and plasma proteins. Amer. J. Obstet. Gynecol. 111: 925-929, 1971.

Serial No.: NIEHS-PP-028
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Induction of Resistance to Pesticides in Cultured Lymphoma Cells:

PREVIOUS SERIAL NUMBER: NIEHS-CB-028

PRINCIPAL INVESTIGATOR: Judson W. Spalding, Ph.D.

OTHER INVESTIGATOR: George W. Lucier, Ph.D.

COOPERATING UNIT: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	1.5
Professional:	1.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is to develop mammalian cell lines in culture which are resistant to selected drugs and environmental agents, to determine the mechanism by which the resistance is expressed and to compare resistant cells with normal cells in terms of appropriate biochemical and metabolic parameters.

METHODS EMPLOYED: Mouse lymphoma cells of the L 5178Y line have been subjected to chronic treatment with gamma chlordane, p, p' DDT, 4-chlorobiphenyl and other agents. This treatment has resulted in the selection of cells which exhibit resistance to the particular agent to which they were chronically exposed. Cloning techniques have been employed to isolate resistant lines originating from single resistant cells.

MAJOR FINDINGS: A cross resistance to different classes of organochlorine compounds (pesticides) has been found to be characteristic of lymphoma cell strains that were originally selected according to their resistance to a specific representative compound, e.g., gamma chlordane, p, p' DDT or 4-chlorobiphenyl. These resistant strains show no significant resistance to several non-organochlorine agents which are toxic to susceptible cells.

The DDT-resistant cell strain (RU-1) has been studied most intensively in order to determine the mechanism of resistance. It has been previously shown that resistance to DDT toxicity by the RU-1 strain is not expressed via any detectable detoxification mechanism. Nor was there any indication that the parent DDT

molecule was being structurally altered in any way by either susceptible or resistant cells.

In collaboration with Dr. George Lucier, it was discovered that the RU-1 cell line had a 15-fold greater level of UDP-glucuronyltransferase (UDP-GTase) activity than did the control (susceptible) cell line. However, this increased level of UDP-GTase activity did not confer any apparent advantage to the RU-1 cell strain as α-naphthol, a substrate of the enzyme, was toxic to the same degree in both resistant and susceptible cells. The significance of the 15-fold increase in UDP-GTase activity in the RU-1 strain is currently under investigation.

While the mechanism of DDT toxicity in lymphoma cells is not understood, current work suggests that the DDT toxicity is being expressed at the level of the cell plasma membrane. Therefore both a qualitative and quantitative characterization of the phospholipid profiles in susceptible and resistant cell strains is under current investigation. The profiles of the four major phospholipid components, phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine, have been characterized in both susceptible cells and the RU-1 strain. No significant differences have yet been indicated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of drug resistance in whole animals depends, at least in part, on the induction of microsomal enzyme systems in the liver. Modification of the toxic molecule is usually followed by conjugation and subsequent elimination.

Resistance exhibited by the DDT-resistant and chlordane resistant cells appears to depend on a different mechanism than that found in the microsomal enzyme system of liver, lung and other tissues. Thus these cells, which do not have the usual microsomal detoxification system, are useful for studying alternative mechanisms of resistance. These studies will also lead to the understanding of how DDT and other highly water-insoluble organchlorine compounds effect their toxicity in biological systems.

PROPOSED COURSE: Efforts will be continued to determine and compare the structural and/or biochemical basis of pesticide resistance among the different resistant cell lines. The significance of the 15-fold greater level of UDP-GTase activity in the (RU-1) DDT-resistant cell line will be investigated further.

The lipid characteristics of membrane systems in susceptible and resistant cells will be compared with emphasis given to the rate of synthesis and subsequent turnover of specific phospholipid species. The nature of resistance will also be studied in relation to phases of the cell replication cycle where indicated.

PUBLICATIONS

Spalding, J.W., Ford, E., Lane, D. and Blois, M.: The character of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane resistance in mouse L 5178Y lymphoma cells. Biochem. Pharmac. 20, 3185-3196, 1971.

Serial No.: NIEHS-PP-029
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Cytotoxicity of Alternaria Toxins to Cultured Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-CB-029

PRINCIPAL INVESTIGATOR: Judson W. Spalding, Ph.D.

OTHER INVESTIGATOR: Ronald W. Pero, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.75
Professional:	0.75
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To determine the degree of toxicity of Alternaria toxins to cultured mammalian cells and to determine the mechanism of action by which the toxicity is expressed.

METHODS EMPLOYED: Alternaria toxins are tested for toxicity to Hela cells in culture and the ID_{50} dose determined. Appropriate concentrations of the compound are selected to determine more specific effects on major metabolic system, e.g., DNA, RNA, protein and membrane synthesis, during defined stages of the cell replication cycle.

MAJOR FINDINGS: The toxicity of alternariol (A-OH), and several other dibenzo-α-pyrone derivatives, has been studied in both Hela and mouse L 5178Y lymphoma cells. The ID_{50} concentration of A-OH is 6.0 ± .5 µgm/ml (ppm) in both cell lines. Complete inhibition of cell replication is achieved at a concentration of 20 µgm/ml.

A-OH apparently has a selective effect on two phases of the cell cycle. Experiments in Hela cells suggested that the initiation of the G-2 phase of the cell cycle was especially sensitive to A-OH and that the toxic effect is reversible and treated cells have been shown to retain their viability through several generations. It was concluded from this data that A-OH was acting at the S/G-2 boundry of the cell replication cycle. However, more recent findings suggest that an inhibition of DNA synthesis in late S-phase cannot be entirely ruled out.

A-OH has also been shown to inhibit cytoplasmic cytokinesis in mouse L 5178Y cells which have been released from an induced state of metaphase arrest during the mitotic phase of the cell replication cycle. Nuclear division continues uninhibited under these same conditions, even though cytoplasmic cytokinesis does not take place. The net result is the occurrence of a high incidence of binucleated cells.

These results indicate that A-OH is acting on some mechanism implicated in facilitating the late anaphase or telophase stages of mitosis. Initial experiments suggested that RNA synthesis was being effected by A-OH during the latter stages of mitosis. However subsequent work has shown that recovery from metaphase arrest does not require de novo RNA synthesis, for the recovery process is not sensitive to Actinomycin D, even though RNA synthesis is completely inhibited. Alternatively, though A-OH does inhibit incorporation of labeled precursor into RNA by 70%, recovery of cells from metaphase arrest is inhibited by nearly 85%. Additional experiments have indicated that recovery from metaphase arrest is relatively insensitive to protein synthesis inhibitors. A-OH has been shown to have little effect on total cell protein synthesis.

When the supply of several other dibenzo-α-pyrone derivatives permitted, experiments were undertaken to investigate the synergistic properties of the related mycotoxins. Alternariol (A-OH), alternariol methyl ether or altenuene did not prove to have a synergistic effect when applied together in various concentrations and combinations in Hela cell cultures.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Toxigenic Alternaria species are commonly found on many foods available for human consumption as well as in feed products for domestic animals. Crude Alternaria extracts have been shown to be toxic to mice and rats as well as to bacterial and tissue culture cells. Several Alternaria toxins, A-OH and A-ME, are toxic to fungi, bacteria, tissue culture cells and mammals (Project Number NIEHS-CB-034, 1970-1971). The determination of the mechanism of toxicity in tissue culture cells is pertinent to the understanding of toxic responses in the intact animal.

PROPOSED COURSE: New toxins from Alternaria will be tested for biological activity as they become available.

Investigation will be continued to determine the mechanism by which the toxicity of A-OH is expressed, especially with respect to its specific activity at the S/G-2 boundry in Hela cells and the late stages of mitosis in mouse L 5178Y lymphoma cells.

Serial No.: NIEHS-PP-030
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Development of a Semi-Micro Assay for Mycotoxin Toxicity in Tissue Culture"

PREVIOUS SERIAL NUMBER: NIEHS-CB-030

PRINCIPAL INVESTIGATOR: Judson W. Spalding, Ph.D.

OTHER INVESTIGATOR: Ronald W. Pero, Ph.D.

COOPERATING UNIT: Research Services Branch, NIEHS

MAN YEARS

Total:	0.5
Professional:	0.0
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: To develop a sensitive, semi-micro assay system for measuring the toxicity of purified mycotoxins in a mammalian tissue culture system.

METHODS EMPLOYED: Tissue culture cells are to be grown on a surface in 1.0 ± 0.5 ml cultures. Rates of macromolecular synthesis measured by the incorporation of the appropriate radioisotopically labeled precursor, will be used as an index of cell growth. The toxicity of any particular mycotoxin or growth inhibitor may then be reflected in the relative inhibition of radioisotope incorporation. In this way, mycotoxins may be compared with respect to their effect on DNA, RNA, protein and lipid synthesis.

MAJOR FINDINGS: An assay system employing the use of tissue culture cells in one ml volume cultures has been developed. The major end-point of the assay concerns the incorporation of ^{3}H-thymidine into DNA. The cell cultures, which are grown on small cover slips, can be easily processed *in situ* and the relative degree of isotope incorporation determined as an index of growth. If a compound of interest, e.g., a mycotoxin, carcinogen, food additive or contaminant, etc., is toxic, the toxicity is reflected in diminished cell growth and subsequently a significantly lesser degree of isotope incorporation into DNA. The effects of a toxic agent on other major metabolic or synthetic pathways may be determined in a similar manner by the selection of the proper isotopically labeled precursor.

PROPOSED COURSE: This project has been successfully terminated and the results are being readied for publication.

PUBLICATIONS

Spalding, J. W., Pero, R. W. and Owens, R. G.: Inhibition of the G_2 phase of the mammalian cell cycle by the mycotoxin alternariol. J. Cell Biology 47: Number 2, Part 2, 1970.

Spalding, J. W., Ford, E., Lane, D. and Blois, M.: The character of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane resistance in mouse L 5178Y lymphoma cells. Biochem. Pharmac. 20: 3185-3196, 1971.

Serial No.: NIEHS-PP-031
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Cytotoxicity of the Mycotoxin 'Patulin' in Cultured Mammalian Cells"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Judson W. Spalding, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.75
Professional:	0.75
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To determine the degree of toxicity of patulin in cultured mammalian cells and to determine the mechanism of action by which the toxicity is expressed.

METHODS EMPLOYED: Patulin was obtained in very high purity and tested for its level of toxicity in mammalian tissue culture cells. Appropriate concentrations of the compound are selected to determine more specific effects on major metabolic or synthetic pathways, e.g., DNA, RNA, protein and lipid synthesis, during defined phases of the cell replication cycle.

MAJOR FINDINGS: Patulin (mol. wt. 154) was found to be extremely toxic to mouse L 5178Y lymphoma cells. The ID_{50} concentration was 0.04 ± 0.1 µgm/ml (ppm) or approximately 2.5×10^{-7}m. The growth of cells was inhibited 100% by 0.08 µgm/ml or higher concentrations.

An initial investigation has indicated that patulin does not dramatically inhibit in vivo DNA, RNA or protein synthesis at relatively high concentrations, 0.05 and 0.10 µgm/ml. Cells in induced stages of synchrony were not inhibited significantly during the S phase of the late stages of the mitotic phase of the cell replication cycle.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Patulin is a carcinogenic lactone of relatively small molecular weight, 154,

and is a metabolite of several species of Penicillium and Aspergillus which have been isolated from food stuffs prepared for human consumption. The chemical structure of patulin is 4-hydroxy-rH-furo[3,2c]pyran-2(6H)-one.

Originally an interest in patulin occurred because it was considered to have potential as a broad spectrum antibiotic. Patulin was found to be toxic in varying degrees to both gram negative and gram positive bacteria, and to some protozoans; it was also shown to have fungicidal activity. However, patulin was later found to be carcinogenic in rats and mice. Its relatively high degree of cytotoxicity and its carcinogenic properties make patulin worthy of study; it is potentially a health hazard in both livestock and humans, especially with reference to the consumption of moldy feeds and fermented food stuffs, respectively.

PROPOSED COURSE: The cytotoxicity of patulin will be compared in other tissue culture systems.

Investigations on the effect of patulin on several major synthetic pathways, e.g., DNA, RNA and protein synthesis, will be extended and clarified. In collaboration with other investigators, the effect of patulin on membrane transport and the major respiratory pathways will be determined.

PHARMACOLOGY TOXICOLOGY BRANCH

PHARMACOLOGY AND TOXICOLOGY BRANCH
Summary Statement

The Pharmacology and Toxicology Branch carries on research in 4 major areas: (1) Toxication-Detoxication Mechanisms, (2) Lung Biochemistry and Pharmacology, (3) Inhalation Exposures and Toxicology, and (4) Reproduction and Ontogenesis. In all research programs our goals remain those of assessing potential toxic effects of pollutants or environmental components, or the interactions of these with other materials to which we are exposed, such as chemicals, food additives, or drugs. Effects seen are studied with regard to their specificity (species, strain, age, or sex of the animal), probable occurrence (at reasonable concentrations or durations of exposure), and mechanisms (with special emphasis on understanding enough of the mechanism to perhaps predict, alter, or prevent serious effects that are discovered experimentally). For example, programs in toxication-detoxication mechanisms have concentrated on uptake and metabolism of chemicals and drugs in the lung and how this uptake and metabolism changes just after birth or is affected by drugs or pollutants to which the animal (baby or adult) is also exposed. We are comparing results in several species, testing pollutants and pesticides over a wide concentration range and for single vs. multiple exposures, and attempting to identify a few of the most potent and long lasting effectors for more detailed studies on mechanisms.

Since last year, major changes in facilities and new personnel have allowed for entirely new research programs in inhalation exposure, and reproduction and ontogenesis. Our new inhalation facility was made available in the summer and new labs for the reproduction research were remodeled early in 1972.

Program

Toxication-Detoxication Mechanisms: We have been comparing detoxication-toxication mechanisms in lung and liver of rabbits and rats and studying how these systems develop with age and respond to exposures of animals to chemicals, pollutants, and/or drugs. The liver has long been thought to be the key organ in the body's metabolism of xenobiotics and steroids. It is becoming obvious that other organs contain systems capable of metabolizing and thereby altering the reactivity of chemicals (and drugs) delivered to them. Our programs are now concerned primarily with determining what kinds of chemical metabolizing systems are present in lung vs. liver and how these chemical metabolizing systems may be involved in removing or making more toxic those chemicals that are ingested by oral or inhalation routes. Chemicals and drugs (including pesticides) often affect liver toxication-detoxication systems and thereby change their own and other chemical's actions and toxicities for the liver and the whole animal. The same is probably true in the lung, but

our work so far suggests that there will be marked differences in kinds of chemicals metabolized in lung vs. liver as well as marked differences in effects of chemicals on these metabolizing systems in lung vs. liver. Therefore, drug-chemical-pesticide-pollutant interactions in lung will produce quite different changes in action and toxicity of a chemical in the lung as compared with the liver.

Lung Biochemistry and Pharmacology: These programs are of quite different types. In one laboratory we have developed a lung perfusion apparatus whereby we can study the uptake and release of chemicals, pollutants, and drugs delivered to the isolated, perfused lung by blood or by inhalation. By such experiments we can more carefully define the parameters influencing uptake, release and interactions of chemicals in the lung since we will not be dealing with indirect effects of the rest of the animal's body on this system. A large number of chemicals are concentrated in the lung - we are limiting our early studies to lung uptake of basic chemicals such as amines. A number of pollutants and pesticides are in this class as are many very important drugs. We are particularly concerned with whether drugs will impede uptake or increase release of pesticides which localize in the lung or vice versa. Also whether this uptake and release are active or passive processes and whether there is a single or multiple process involved. The localization of basic chemicals in the lung is important to the duration of action and toxicity of many of these chemicals. Yet little is known about this process or interactions of chemicals at this process.

Another research group is especially concerned with the biosynthesis and release of lung surfactant and the changes in these processes with age and with exposure to pollutants. Lung surfactant seems to play a key role in lung pathology in the newborn and in the processes leading to emphysema, and studies of the key steps in the synthesis of this material may help in altering that process or preventing its destruction by toxic materials. Another program in this laboratory concerns the role of lung lipids in the storage of fat soluble pesticides and chemicals such as DDT and PCB. This program also interdigitates with a similar effort concerned with pesticide and pollutant uptake by liver lipids and especially the liver lipid body. Storage of fat soluble pesticides and pollutants in lung and liver probably regulates not only the duration of their stay in these organs, but their toxic effects in these and other tissues. Kinetics of uptake and release of these materials in lung and liver lipids are being studied in hopes of understanding how these processes may be altered.

Inhalation Exposures and Toxicology: Work here has been delayed by late delivery and installation of exposure chambers. Studies performed so far include (1) the effects of repeated exposure to SO_2 on the ability of lung and liver to detoxify SO_2 by oxidation to SO_4^{-2}, (2) effects of single and repeated exposures to benzene or xylene in rats also treated with drugs and chemicals that change benzene or xylene detoxication in liver and lung, and (3) effects of single and repeated exposures of rats and rabbits to selected components of aerosol deodorants. Our preliminary results indicate that the collaborative efforts between the inhalation toxicologist and scientists in the Toxication-Detoxication Mechanisms and Lung Biochemistry and Pharmacology programs have

been most productive. The most interesting of our studies show that interactions of chemicals and drugs can markedly alter the toxicity of materials inhaled by animals - thus xylene toxicity is markedly shifted by previous exposure of the animal to chemicals and drugs that affect liver and lung metabolism by xylene. We have also seen that inhalation of hexachlorophene or aluminum chlorhydrin (major components of aerosol deodorants) can affect the lung toxication-detoxication systems at exposures that have little or no histologically observable effects on the lung - i.e. - the lung detoxication systems may serve as early warning systems for damage as well as explaining some interactions that are puzzling to classical toxicology.

Reproduction and Ontogenesis: Remodeling labs and equipping them for studies in this area were completed only after Christmas. Work so far has been directed towards setting up the technics for collecting and storing ova, sperm, and fertilized ova from several species. This section is also able to culture fertilized ova in vitro for several days and reimplant such embryos (having grown in culture to this stage) in foster mothers (pseudo-pregnant) and then subject the newborn animals to examination for teratolgoy, etc. These technics will allow us to study effects of precisely defined environmental alterations on reproductive processes up to and slightly beyond the time of placentation. Precisely known amounts of known chemicals or pollutants can be added to the culture or injected into the egg or embryo at known times. Technics are also being developed to transfer nuclei from such treated cells into normal cell cytoplasms so as to decide where a chemical has had its effect (nucleus or cytoplasm). Such technics have not been applied to understanding teratology of chemicals or environmental pollutants anywhere else to our knowledge and will allow us to delineate more clearly than before what the teratogen is and how it acts. This elegant type study will be coupled with routine, classical investigation of chemicals and mixtures for teratologic effects in animals. To help us in these studies we hope to initiate a contract for some such screening studies since the present staff is inadequate for such efforts except on an extremely limited basis. Hopefully this section will not have to restrict its efforts only to teratology. Environmental effects on the entire process of ontogenesis are to be studied in the future as staff, etc., become available. Environmental pollutants undoubtedly affect the aging process too and this will be studied as resources permit. Our initial efforts are focused on early gestation at present only because this is the "best bet," not because we wish only to study this time of life.

Facilities

Inhalation facilities are being developed. At present we have 4 small chambers and 4 large chambers installed and operating. We will soon have 4 isolation chambers ready, two of which will allow us to study highly toxic or suspected carcinogenic substances in small animals (rats, hamsters). We have purchased equipment to outfit about one-half of the rooms in Building 14 and hope to complete installation of this by Fall 1972. In the next year we hope to outfit two to three more rooms so as to carry on a few chronic inhalation experiments which cannot now be well done. A main drawback to efficient use of this exposure facility is the lack of manpower to operate the chambers.

Dr. Drew's group is in charge of this unit and to operate the 10 rooms soon to be ready will require at least 15 people. At present Dr. Drew has four technicians, one Staff Fellow, and one Visiting Fellow.

Personnel

Personnel added since the last progress report include: Dr. Thomas Eling (lung perfusion), Dr. Roland Roth (inhalation toxicology), Dr. Laila Moustafa (reproduction), and Dr. Richard Philpot (lung detoxication systems) at the professional (Staff Fellow) level. Technical and support staff additions included Mrs. Sue Garrett (replaced Branch Chief's secretary), Mr. Douglas Parkes (with Dr. Eling), Mr. Clyde Williams (with Drs. Hook and Bend), Mrs. Carol Sloan and Mr. Robert Kellam (with Dr. Staples), and Mr. Mike Riley (with Dr. Drew). Part-time, temporary, and stay-in-school staff additions included Mrs. Barbara Gowan (secretary, part-time), Mr. Ray Simmons (technician, temporary), Mr. Frank Schwartz (technician, temporary), Mr. James Abron (stay-in-school), Mr. Louis Hassell (stay-in-school), Miss Patricia Finger (stay-in-school), Miss Octavia Walters (stay-in-school), and Mr. Bruce Woodin (stay-in-school). Visiting Fellows who have recently arrived included Dr. Terry Orton (with Dr. Fouts), Dr. Ray Lo (with Dr. Drew), and Dr. Gerald Stranzinger (with Dr. Staples).

At present then, the Branch is staffed overwhelmingly by non-tenured people, a large proportion of which are not even full-time. Although this arrangement is the best possible under present limits and conflicting personnel programs, it is hardly a desirable way to construct an even moderately future-oriented or continuing research effort.

Other Activities

Several of our professional staff serve on committees, etc., helping other organizations such as the local universities, other Institutes at NIH and other Governmental agencies, or professional societies. The listing below is meant to be complete, but undoubtedly suffers some omissions.

Dr. Fouts is an Adjunct Professor of Pharmacology in the School of Medicine, University of North Carolina, Chapel Hill, and lectures in the graduate school and medical school courses in pharmacology. He is a member of the Dental Research Center's Scientific Review Committee (UNC, Chapel Hill) and has served on three subcommittees of that body this year. He is also an Adjunct Professor of Toxicology in the School of Agriculture and Life Sciences, North Carolina State University at Raleigh and serves on the Toxicology Advisory Committee of the Department of Entomology at that University. He is a member of the editorial boards of the journals: Chemico-Biological Interactions and Xenobiotica. He is a member of the Education Committee of the Society of Toxicology and the American Society of Pharmacology and Experimental Therapeutics Committees on Environmental Pharmacology and Public Information (this latter for the Fifth International Congress on Pharmacology). He is a member of the Committee on Anticonvulsant Drugs of the National Institute of Neurological Diseases and Stroke (NINDS, NIH) and the Scientific Advisory Board of the National Center for Toxicological Research, University of

Arkansas and the Food and Drug Administration (FDA, DHEW). Dr. Fouts was also an invited speaker at the Gordon Research Conference on Drug Metabolism (July 26-30, 1971), the International Symposium on Pharmacology and Anticonvulsant Drugs (Phoenix, September 7-10, 1971), the 13th International Congress of Pediatrics, Vienna, Austria (August 29-September 4, 1971), and the International Symposium on Fetal Pharmacology, Stockholm, Sweden (December 13-15, 1971). Dr. Fouts is on several NIEHS committees - e.g. - Contract Review, Safety, Library. He has also served on ad hoc contract reviews for NIGMS, NIH.

Dr. Robert Drew is a member of the Environmental Health Committee of the Society of Plastics Industries and an Advisory Committee to the lung cancer segment of the chemical carcinogenesis group at the National Cancer Institute (NCI, NIH). Dr. Drew and Dr. Laskin (at NYU) wrote a chapter "Environmental Inhalation Chambers" in the book Methods of Animal Experimentation, Vol. 4, Academic Press, N.Y., in press.

Dr. Robert Staples is a member of an ad hoc contract review group of the Contraceptive Development Branch, Center for Population Research, National Institute of Child Health and Human Development (NICHD, NIH). He also wrote a chapter "Teratology" in the book Pharmacology of Antiepileptic Drugs, Raven Press, N.Y., in press.

Dr. Theodore Gram is an Adjunct Assistant Professor of Pharmacology in the School of Medicine, UNC, Chapel Hill. He is also a member of the editorial board of Biochemical Pharmacology and has served on a Contract Review Committee of the Laboratory of Chemical Pharmacology of the National Cancer Institute (NCI, NIH). He is a member of the Editorial Committee of NIEHS.

Serial No.: NIEHS-PT-001
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Enzymes Metabolizing Chemicals: Chemical Effectors of These Systems (Inducers, Inhibitors, Stimulators)"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: James R. Fouts, Ph.D.

OTHER INVESTIGATORS: Richard Philpot, Ph.D., Henn Kutt, Ph.D., Alfred R. Hansen, Ph.D., and R. J. Pohl, B.S.

COOPERATING UNITS: Biometry Branch

MAN YEARS:

Total:	0.6
Professional:	0.2 (plus 0.5 for Kutt and Hansen)
Other:	0.4 (plus 0.2 for Waters)

PROJECT DESCRIPTION

OBJECTIVES: This is a general program directed to classifying and understanding the actions of chemicals on body mechanisms for metabolizing "xenobiotics" and other substances (endogenous as well as exogenous) which can be substrates for systems often called "drug metabolizing enzymes." Such enzyme systems can be found relatively concentrated in several excretory organs or in organs found at the interface between man and his environment (liver, lung, gut, kidney). These enzyme systems respond to (are affected by) a variety of chemicals to which man and animals are exposed in their environment such as pesticides, pollutants, and metals. Chemicals that affect these metabolizing systems may act differently at different doses, by different routes of administration, or at different times after administration. Combinations of chemicals often produce effects quite different from single entities used alone. The present study continues research on metal and metal ion effects on these metabolizing systems in the liver and on metal ion-inducer-xenobiotic substrate interactions in liver and lung. Also described are some projects started at the University of Iowa and finished at NIEHS dealing with diphenylhydantoin and benzpyrene.

METHODS EMPLOYED: Microsomes are isolated from livers or lungs of control animals or animals treated with chemicals which stimulate or induce microsomal enzymes that metabolize xenobiotics. Studies are then made of xenobiotic metabolism by these microsomes or of effects of metal ions, buffer, pH, ionic strength on the activity of two microsomal electron transport systems: NADPH-

cytochrome c reductase, and NADPH-cytochrome P-450 reductase. The xenobiotic metabolisms are followed by classical technics. Chemical-microsomal interactions can also be studied spectrally; substrate-induced difference spectra. The cytochrome reductase activities are being studied spectrophotometrically in the Aminco-Chance using the Aminco-Morrow stop-flow attachment so as to obtain better estimates of the initial rates of these rapid reactions. The substrate-induced difference spectra can be studied on either the Shimadzu or Aminco-Chance spectrophotometers.

MAJOR FINDINGS: Divalent cations can affect both hepatic and lung microsomal NADPH cytochrome c and P-450 reductase activities. The most complete studies have been made with hepatic enzymes, but no qualitative differences have yet been seen between metal ion effects on liver and lung cytochrome reductases where these have been compared. Divalent cation effects can be either stimulating or inhibiting and the same cation can have both effects at different concentrations. Divalent cations act at relatively low concentrations, but maximal effects may not be reached before 50-100 mM. The major findings concerning effects of Mg^{+2} on hepatic NADPH cytochrome P-450 reductase and the interactions of Mg^{+2} with substrates and hepatic microsomes from 3-methylcholanthrene (3-MC) or phenobarbital pretreated rats are described in a paper published after the last annual report (see below). Work during the past year has concentrated on the ability of buffers and monovalent cations to block the stimulating or inhibiting effects of divalent cations on these reductases. Monovalent cations and buffers (ionic or "non-ionic" such as HEPES) are only slightly stimulating to P-450 reductase, yet when present in large quantities can block the actions of divalent cations like Mg^{+2}. Monovalent cations and buffers can also block the stimulating effects of chemical substrates on P-450 reductase. Microsomes from 3-MC-treated animals are less responsive to all stimulators - metals, substrates, buffers, and ionic strength and this is not due just to presence of residual amounts of the inducer (3-MC) in microsomes. We are trying to purify P-450 (P-448) from inducer-pretreated rats to see if metal ion interactions with this purified system are different from the intact microsomes as well as whether P-450 and P-448 react differently with metal ions and substrates in terms of rates of reduction by NADPH. This is a joint project with Dr. Philpot.

Two other projects continued and "finished" work begun at the University of Iowa just before coming to NIEHS. One of these was with Dr. Henn Kutt and Mr. L. Waters; the other was with Dr. Alfred Hansen.

In the project with Dr. Kutt we studied the effects of pretreating rats with drugs (diphenylhydantoin or DPH, phenobarbital) or pesticides (DDT) or pollutants (3-methylcholanthrene) on substrate-induced difference spectra with microsomes from these animals. Comparison was also made with effects of adding these "inducers" (DPH, phenobarbital, DDT or 3-MC) in vitro to control microsomes vs. use of them only in treating the animal before sacrifice and isolation of microsomes for spectral studies. Most of the interactions seen could be explained on the basis of interactions between the chemicals at the cytochrome P-450 binding sites. Thus many of the changes in microsomal difference spectra that appear to result from enzyme induction may be associated with presence of residual inducer in these microsomes. The only exception to this was effects of 3-MC on aniline-induced difference spectra where the 3-MC pretreatment of

the animal appeared to create (induce) a new binding site not seen merely by adding 3-MC in vitro to control microsomes.

In the project with Dr. Hansen we studied the enzyme benzpyrene hydroxylase and some problems in its assay after rats were pretreated with inducers of that system (such as 3-MC). We found that the primary metabolites of benzo(α)pyrene appear to disappear with continuing time of enzyme action, especially with microsomes from induced animals. We found that even very short incubation times (less than 2 minutes) could not be used for estimates of initial reaction velocity when kinetics experiments were done with substrate concentrations in the range of Km (from 1/2 to 5 times Km), but only if substrate was kept greatly in excess of Km (10X to 20X Km). We found that benzpyrene substrate could be added to incubation mixtures in suspension in Tween 80 rather than complexed with albumin or other protein whose binding of substrate might confound kinetics studies. Finally we showed that fluorescent spectra of benzpyrene metabolites at the beginning of the metabolism (time 0 to 2 minutes) are different from those remaining after the initial phase is over (time 5-15 minutes). These findings indicate that benzo(α)pyrene hydroxylase will be an extremely difficult enzyme to study especially in the induced rat and that the problems involved are not solved by the currently used assays for this system. These results have been submitted to the journal Chemico-Biological Interactions.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolism of xenobiotics by enzymes in excretory organs or tissues exposed to the environment (liver, lung, gut) can affect the toxicity of these chemicals. This metabolism is also affected by many environmental contaminants - being stimulated or blocked by a variety of pesticides, pollutants, and metals. The specific study of metal ion effects on these systems in liver and lung seeks to understand normal control mechanisms as well as effects of exposures to chemicals that change metal ion concentrations in cells (chelators), affect metal ion interactions with these systems by changing the systems (inducers such as 3-MC), or are themselves metal ions (heavy metals) which can act directly. P-450 reductase is believed to be a key rate-limiting step in chemical metabolizing systems in liver and we hope to shed some light on its role in the lung toxication and detoxication mechanisms. Metal ion effects on chemical metabolisms have been seen both in vivo and in vitro and our studies seek to extend these findings to a molecular level as well as to pinpoint the most sensitive step in the overall process and thereby to indicate where changes will be most possible and effective as therapy or antidotes. The studies on pesticide, etc., effects on microsomal difference spectra (with Dr. Kutt) distinguish direct from indirect effects of pesticides, etc., as inducers and effectors of microsomal binding sites for chemicals. The project with Dr. Hansen established firmly a serious problem in assays of benzpyrene hydroxylase - namely that the enzyme from induced animals cannot be studied with most of the currently used methods. The metabolism of benzpyrene by the microsomal systems (Hansen project) and the effects of chemicals and pesticides on microsomal binding sites (Kutt project) are related to the overall toxicity and interactions of these effectors and substrates of the microsomal systems.

PROPOSED COURSE: Continue comparison of lung with liver systems especially after treatement of animals with polycyclic hydrocarbons. Try to isolate P-450

(P-448) from lung and liver of control vs. 3-MC treated animals to better indicate the kind of changes in metal-substrate-cytochrome interaction which may explain the differing reactivity or non-reactivity of the induced cytochrome and its reductase. Further screening for effects of heavy metal ions and trivalent ions, especially aluminum effects on lung P-450 reductase in conjunction with Dr. Drew's studies on lung damage by aluminum-containing deodorant sprays.

PUBLICATIONS

Fouts, J.R. and Pohl, R.J.: Further studies on the effects of metal ions on rat liver microsomal reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase. Journal of Pharmacology and Experimental Therapeutics. 179: 91-100, 1971.

Kutt, H., Waters, L., and Fouts, J.R.: The effects of some stimulators (inducers) of hepatic microsomal drug-metabolizing enzyme activity on substrate-induced difference spectra in rat liver microsomes. Journal of Pharmacology and Experimental Therapeutics. 179: 101-113, 1971

Fouts, J.R. and Kutt, H.: Some studies on the biotransformation and interactions of diphenylhydantoin with some other drugs and chemicals. In Penry, Woodbury, et al (Ed.): Pharmacology of Antiepileptic Drugs. Raven Press, New York, 1972, (in press).

Hansen, A.R. and Fouts, J.R.: Some problems in Michealis-Menten kinetic analysis of benzpyrene hydroxylase in hepatic microsomes from polycyclic hydrocarbon-pretreated animals. Chemico-Biological Interactions. In press, 1972.

Serial No.: NIEHS-PT-002
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Enzymes Metabolizing Chemicals; Physiological Effectors of these Systems (age, species, sex, nutrition)"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: James R. Fouts, Ph.D.

OTHER INVESTIGATORS: R.S. Chhabra, Ph.D., T. Devereux, M.S., and R. Pohl, B.S.

COOPERATING UNIT: Biometry Branch

MAN YEARS:

Total:	2.4
Professional:	1.2
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVES: This is a general effort directed toward classifying and understanding various factors that can affect xenobiotic metabolism by various tissues. These studies will concern the effects of age, strain, sex, or species of animal on metabolism by liver and lung (and other tissues) of xenobiotics. Current projects concern the species differences in stimulation of hepatic xenobiotic metabolism by DDT and benzpyrene (mice vs. rats, Dr. Chhabra); possible sex differences in xenobiotic metabolism by extrahepatic tissues in the rat (lung, gut; Dr. Chhabra); and metabolism of xenobiotics in liver vs. lung in the first month of life (Mrs. Devereux and Mrs. Pohl).

METHODS EMPLOYED: Liver (or other tissue such as lung, gut) is isolated from control (or various ages of animals) or treated animals (treated with differing doses of DDT or benzpyrene for differing periods of time and/or by differing routes) or animals of different sex or species (rat vs. mice vs. rabbit). The tissues are then treated to remove as much blood or food (gut) as possible (perfused, washed, or minced with KCl), the more active parts retained (cut out gall bladders, discard trachea, scrape out mucosa), macerated and homogenized and subjected to differential centrifugation to recover microsomes where this is needed. Xenobiotic metabolism can be studied with homogenates or with isolated subcellular particulates and substrates chosen include examples of type I (benzphetamine, benzpyrene, and aminopyrine), type II (aniline), and undetermined classes (p-chloromethylaniline, p-nitro-anisole).

Spectrophotometric methods are used to study cytochromes (b_5, P-450), reductases (P-450 and cytochrome c reductases), and difference spectra (induced by adding substrate to microsomes).

MAJOR FINDINGS:

1. Dr. Chhabra's projects: We have found that mice are relatively resistant to the hepatic microsomal enzyme inducing effects of DDT and benzpyrene as compared with rats. This effect, first seen at Iowa, has been confirmed here. The resistance is only relative however, and mouse liver enzymes will respond to either DDT or benzpyrene if massive doses (given i.p.) are used (100 mg/kg and higher) and especially if repeated several times. Also important is the duration between last dose of DDT or benzpyrene and time of sacrifice of animal for enzyme assay. In rats the maximal effects of both inducers are relatively longer lived (at least 72 hours with benzpyrene and often more than a week with DDT). In mice the "induction" is lost quickly - somewhere between 24-48 hours with both inducers.

Studies of male vs. female rats have shown livers from males metabolize most xenobiotics at rates 2-3 times greater than females. We have studied lung metabolisms in rats and find no such sex differences. We are just starting a study of gut xenobiotic metabolism.

2. With Mrs. Devereux and Mrs. Pohl - Xenobiotic metabolism in lung as function of age: We have completed the first part of this study in rabbits - comparing xenobiotic metabolism and cytochromes at four ages - 3-5 days, 14 days, 30 days, and adult (4-6 mos) - in liver and lung. Two patterns emerge - a slow steady increase up to 30 days when nearly adult activity is seen (most lung systems) or a sudden jump between 14 and 30 days (most liver systems). Most lung microsomal systems are in much lower concentrations or levels at all ages than in liver microsomes. Exceptions are NADPH-cytochrome c reductase and benzphetamine demethylase where both lung and liver microsomes are equally active at all ages (but where there is a progressive increase in level with age in each tissue).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species differences in response of xenobiotic metabolizing systems to exposure to pesticides and pollutants can markedly affect interactions of these pesticides, etc., with other chemicals and drugs as well as be at the root of species differences in pesticide, etc., toxicity. The relative "resistance" of mice (compared with rats) to DDT and polycyclic hydrocarbon effects on liver may alter much interpretation of previous toxicity data using mice.

Studies on the rate of development of xenobiotic metabolism by liver and lung in the newborn can help explain differences in toxicity and interactions of chemicals at these ages vs. adults. These studies are also of great interest to work being done by Dr. Staples and his group working in Developmental and Perinatal Biology.

PROPOSED COURSE: We will study the reasons for mouse resistance to DDT and benzpyrene especially whether the mouse eliminates these chemicals faster than the rat. Studies on sex differences in extrahepatic xenobiotic metabolism

will continue and will help define optimal assay conditions in tissues like gut as well as whether males and females differ in xenobiotic metabolism in these other organs.

Age-related changes in xenobiotic metabolism by lung will be studied in terms of effects of inducers and stimulators at various ages, and whether the rate limiting step might change with age - from reductase to cytochromes to rate of reduction of cytochromes. At present we are also trying to purify cytochrome P-450 from lung and will add this to systems from the newborn to see if the defect might be only a quantitative rather than qualitative one.

PUBLICATIONS

Fouts, J.R.: Maturation and induction of microsomal drug-metabolizng enzymes. In Proceedings of the 13th International Congress for Pediatrics. Vienna, Austria, Verlag der Wiener Medizinischen Akademie, 1971, pp. 99-103.

Fouts, J.R.: Microsomal mixed-function oxidases in the fetal and newborn rabbit. Proceedings of the International Symposium on Fetal Pharmacology. Raven Press, N.Y. (in press).

Fouts, J.R. and Devereux, T.R.: Microsomal enzyme systems in rabbit lung and liver during the first month of life. Toxicology and Applied Pharmacology. 21: , 1972 (Abstract). (In press).

Serial No.: NIEHS-PT-003
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Effects of Chemicals on the Inhalation Toxicity of Chemical Solvents"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. T. Drew, Ph.D.

OTHER INVESTIGATORS: Frank Harrington, Emile Lores, B.S., Roberta Pohl, B.S., Mike Riley, and Joanna Schroeder

COOPERATING UNITS: Pathologic Physiology (Histology), Biometry Branch

MAN YEARS:

Total:	0.7
Professional:	0.3
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: Certain chemicals are known to activate or induce the liver microsomal enzyme systems in the rat. The object of this study is to determine if pretreatment by these chemicals, or by chemicals which may affect the lung microsomal enzyme systems, will alter the acute inhalation toxicity of selected organic solvents. Intraperitoneal injection toxicity studies of the solvents are also being performed for comparison of effect of route of administration on results obtained.

MAJOR FINDINGS: Biochemical methods have been developed measuring the *in vitro* rate of conversion of benzene to phenol. In animals pretreated with phenobarbital, the rate of conversion of benzene to phenol is increased in liver microsomes but not changed in lung microsomes. Animal pretreatement by chlorpromazine increases the rate at which both lung and liver microsomes metabolize benzene but the effect on the liver is not as pronounced as with phenobarbital. In spite of these demonstrable changes in the rate of benzene metabolism, pretreatment by phenobarbital (75 mg/kg) or chlorpromazine (15 mg/kg) does not alter the acute inhalation toxicity or the IP injection toxicity of benzene. Studies with 3-methylcholanthrene show that it also increases the rate of benzene metabolism in the liver but the IP benzene toxicity is not altered.

Inhalation and IP injection toxicity studies have also been completed with p-Xylene. Pretreatment by phenobarbital, 3 methylcholanthene or chlorpromazine

does not alter the IP toxicity of p-Xylene. However phenobarbital does exhibit a protective effect on the inhalation toxicity of p-Xylene. The LC_{50} goes from 4500 ppm in control animals to about 6000 ppm in animals treated with phenobarbital.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In today's society the number of people taking drugs either prescribed by a physician or illicitly is substantial. Some of these compounds stimulate liver microsomal enzymes, and possibly lung systems, thus increasing the rate at which certain chemicals and/or endogenous substrates are metabolized. This experiment is designed to determine if the toxicity of some selected solvents is altered by pre-exposure to certain drugs and whether route of administration affects the results obtained.

PROPOSED COURSE: These relationships, as well as others will continue to be investigated. Additional effort will be directed toward elucidation of the lung and liver metabolism of p-Xylene. The effects of these inducer drugs on the inhalation and injection toxicity of toluene will be investigated.

Serial No.: NIEHS-PT-004
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Analytical Methodology - 1. Model Substrates of Mixed Function Oxidase in Liver and Lung"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. S. Chhabra, D.V.M., Ph.D. and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: T. E. Gram, Ph.D., J. R. Bend, Ph.D., J. R. Fouts, Ph.D., and R. Easterling

MAN YEARS:

Total:	0.9
Professional:	0.7
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: In vitro studies of microsomal xenobiotic metabolisms use various chemicals as substrates. At present these are divided into at least three categories on the basis of spectral studies of mixtures of microsomes with the chemical. Thus reference is made to type I, type II, and modified type II spectra. Representatives of each type of substrate are used in most studies since these appear to react differently to inhibitors, inducers, and stimulators of metabolism as well as to differ from each other as affected by animal age, species, sex, etc. We have devoted time to two assays this year: (1) aniline hydroxylase, and (2) biphenyl hydroxylase. Aniline is a type II substrate and the problem concerned differences in apparent aniline metabolism using a quick method (TCA precipitation) vs. an extraction procedure (ether) with crude (homogenate, 9000 xg supernate) vs. purer (microsome) enzyme preparations. Biphenyl hydroxylase is a substrate of interest for several reasons - (a) as an alternative to the tricky benzpyrene hydroxylase assay (see NIEHS-PT-001), (b) as a substrate whose metabolites are known and quantifiable as such (benzpyrene metabolites are not) and whose metabolites shift with age (ratio of 2 to 4 hydroxy- shifts markedly from birth to maturity). The problems with biphenyl hydroxylase were to optimize the incubation conditions for lung and liver in the rabbit.

METHODS EMPLOYED: Microsomes were isolated from liver and lung of various ages of rats or rabbits and studies of protein, buffer, cofactor, substrate, and time of incubation variations were made. Metabolites (p-aminophenol, 2 and 4-HO-biphenyl) were assayed by classical technics using spectrophotometry and spectrophotofluorometry.

MAJOR FINDINGS: Aniline hydroxylase assays must be made using the ether extraction procedure if assays are to measure the p-aminophenol formed by the MFO systems. The problems arise when soluble fraction is used in the incubation mixture - apparently due to SH groups in that soluble fraction which interfere with the formation of the indophenol dye by which the aminophenol is assayed.

Biphenyl hydroxylase assays have been optimized for both rabbit liver and lung microsomes. Interesting effects of substrate "solubilizers" are seen in terms of overall metabolism as well as kinetics. Liver and lung biphenyl hydroxylase are nearly identical in tissues from adult rabbits. This plus benzphetamine demethylase are the only two xenobiotic metabolisms at similar rates in liver vs. lung.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Model substrates for xenobiotic metabolism assays are needed for several tissues in order to measure this function in these tissues and to study effects of pollutants, pesticides, etc., on this function. Some of the "models" are themselves potential hazards to health and widely used or distributed and their study as such is important (e.g. - benzpyrene, biphenyls).

PROPOSED COURSE: Problems of this nature - methodology for better assessment of xenobiotic metabolism will continue to occupy some fraction of the effort of this section and program.

PUBLICATIONS

Chhabra, R.S., Gram, T.E., and Fouts, J.R.: A comparative study of two procedures used in the determination of hepatic microsomal aniline hydroxylation. Toxicology and Applied Pharmacology. 21: , 1972 (in press).

Serial No.: NIEHS-PT-005
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "A Comparative Study of the Mixed Function Oxidase Systems of Rabbit Lung and Liver with Special Emphasis on the Properties of the Pulmonary Mixed Function Oxidase Systems"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D. and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., C. Williams, M.S., and R. Easterling

COOPERATING UNITS: Biometry Branch

MAN YEARS:

Total:	2.5
Professional:	1.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Since foreign organic chemicals may enter the body via lungs during normal respiration, the metabolism or biotransformation of these xenobiotics by the lung may be important to environmental health. This investigation is directed towards (1) characterizing the microsomal mixed function oxidase (MFO) system of rabbit lung; (2) the identification of the cell type or types in lung which contain the drug metabolizing activity, and (3) the effect of environmental agents, which are known inducers or inhibitors of the liver MFO system, on pulmonary MFO activity.

METHODS EMPLOYED: Microsomes were prepared from homogenized lung by differential centrifugation and enzyme assays were carried out spectrophotometrically.

MAJOR FINDINGS: In general, the protein-activity, time-activity, substrate concentration-activity and pH-activity curves of rabbit lung microsomal aniline hydroxylase, biphenyl hydroxylase, and D-(+)-benzphetamine demethylase are similar to the same enzymes of liver microsomes. Although the Km values for biphenyl hydroxylase and benzphetamine demethylase were not significantly different in liver and lung, the apparent Vmax values were significantly higher in lung in each case even though the lung contains only 20-25 percent as much cytochrome P_{450} (P-450) per mg microsomal protein. However, the initial rate of cytochrome P-450 reductase activity of liver and lung microsomes of the rabbit are of the same magnitude.

A typical type I difference spectrum was obtained when D-(+)-benzphetamine was added to lung microsomes whereas aniline usually produced an atypical type II difference spectrum (having a peak near 430 nm and a long trough from 390-415 nm frequently sharing a minimum at 410 nm).

The lung consists of more than 40 different cell types, some of which are unique to this organ. The alveolar macrophage has been isolated, disrupted by sonication, and a microsomal pellet prepared. These microsomes contained negligible benzpyrene hydroxylase, biphenyl hydroxylase, aniline hydroxylase, or benzphetamine demethylase activity and no apparent cytochrome P-450. However a "b" type cytochrome was noted which was reducible by either NADH or NADPH. Thus, although the actual cellular site of lung drug-metabolizing activity is still unknown, our data indicate that the contribution of the alveolar macrophage is negligible.

Phospholipids are integral parts of the liver microsomal MFO system. Consequently, the effects of phospholipase c (PLP-C) treatment on liver and lung microsomes have been compared. In both lung and liver PLP-C incubation causes a 20-30 percent loss of cytochrome P-450, a 70 percent decrease in microsomal phospholipid and no change in the cholesterol content (per mg microsomal protein). Lung biphenyl hydroxylase and aniline hydroxylase activities were each decreased by about 50 percent on PLP-C treatment whereas the liver activities decreased only 22 percent (biphenyl) and 13 percent (aniline), respectively. Addition of sonicated phosphatidylcholtine to the PLP-C treated microsomes did not restore the activity in liver or lung.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental pollutants that are inhaled come directly into contact with the lungs of the mammalian species involved. Consequently, the ability of the lung to metabolize xenobiotics and the characterization of the enzymes responsible for this metabolism are of interest. There is another consideration which may be even more important, however. Chemical agents which are inducers or inhibitors of the liver and lung MFO systems are known. Inhalation of such effectors of drug metabolism in contaminated air may play a significant role in the *in vivo* detoxication capacity of an individual. Such induction or inhibition of lung enzymes could be especially important if it interferes with normal substrate (rather than xenobiotic) metabolism - e.g. - steroid or phospholipid metabolism.

PROPOSED COURSE: Compounds which are known effectors of the microsomal MFO system of liver will be administered to rabbits by one or more routes (inhalation, tracheal intubation, parenteral, or oral) and lung MFO activities determined. Using a potent inducer of the lung system, the various routes of administration will be compared for their effect on lung MFO. Also enzyme digestion of the lung and centrifugation techniques will be employed in efforts to isolate different cell types from lungs to find which of the many different cells possess the MFO activity.

PUBLICATIONS

Hook, G.E.R., Bend, J.R., Hoel, D., Fouts, J.R., and Gram, T.E.: Preparation of lung microsomes and a comparison of the distribution of enzymes between

subcellular fractions of rabbit liver and lung. Pharmacology and Experimental Therapeutics. (In press).

Bend, J.R., Hook, G.E.R., Easterling, R.E., Gram, T.E., and Fouts, J.R.: A comparative study of the hepatic and pulmonary mixed-function oxidase systems in the rabbit. Pharmacology and Experimental Therapeutics. (In press).

Serial No.: NIEHS-PT-013
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Biosynthesis and Degradation of Lung Surfactant Phospholipids"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: S. Gipson, B.S., and J. Abron

COOPERATING UNITS: None

MAN YEARS:

Total:	1.8
Professional:	0.8
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: A surfactant system located at the lining of the lung appears to be essential for alveolar stability (patency). It is generally believed that this surfactant is chiefly composed of the phospholipid dipalmitoylphosphatidylcholine. The rapid rate of biosynthesis of this phospholipid by mammalian lung parenchyma is apparently vital for the replenishment of surfactant at the alveolar lining. The objectives of the proposed studies are to investigate the biosynthesis and degradation of lung surfactant phospholipids, especially dipalmitoylphosphatidylcholine, and to localize the site(s) for such biochemical events.

METHODS EMPLOYED: Chromatography (TLC and GLC) and liquid scintillation techniques were employed.

MAJOR FINDINGS: 1-^{14}C-palmitate, 1-^{14}C-oleate and ^{32}P were rapidly incorporated into rat lung phosphatidylcholine. The low specific activity and turnover of phosphatidylethanolamine suggests that the CDP-choline pathway may be domnant in the lung for the synthesis of surfactant phosphatidylcholine. One other lung phospholipid, tentatively identified as phosphatidyl-N, N-dimethylethanolamine (PDME), was found to very rapidly incorporate 1-^{14}C-palmitate and had a half-life of about 5 hours. The half-life of oleate in the latter compound was greater than twenty hours. On further study using two-dimensional thin-layer chromatography we can identify little, if any, PDME. The chromatographic properties of this unidentified phospholipid appear to coincide more closely with that of phosphatidylglycerol. Significant amounts of phosphatidylglycerol

appear to be present in crude fractions of the rabbit concentric lamellar body (CLB), which is considered to be the secretory form of lung surfactant. Compositional analyses of the rabbit CLB seem to support this hypothesis. The CLB had a phospholipid/protein ratio greater than 5 with the predominant phospholipid being phosphatidylcholine. Only small amounts of neutral lipids were present.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The secretion and maintenance of a surfactant acellular lining appears to be vital for alveolar surface stabilizations. Abnormally low amounts of surfactant are thought to precipitate alveolar collapse, or atelectasis. This surfactant system of the lung is chiefly composed of dipalmitoylphosphatidylcholine (DPL). Infants suffering from the respiratory distress syndrome, or hyaline membrane disease, where atelectasis is eminent have been shown to have significantly lower levels of lung DPL. It would seem important, therefore, to investigate those factors which contribute to the synthesis, storage, secretion and disposal of lung surfactant phospholipid. This information could also serve in the assessment of potential damage to lung integrity by various ingested or inhaled environmental agents.

PROPOSED COURSE: 1) To continue in attempts to further purify the rabbit CLB and examine the composition and uptake of phospholipid precursors into CLB. 2) To examine the effects of various agents on the formation of CLB and lung phospholipids. Special attention will be given to potential inducers of CLB's or lung phospholipid biosynthesis.

PUBLICATIONS

DiAugustine, R. P.: I. In vivo studies of the incorporation of ^{32}P, [methyl-^{14}C] choline, 1-^{14}C-oleic acid into phosphatidylethanolamine, phosphatidyl-N,N-dimethylethanolamine and phosphatidylcholine. Biochem. Biophys. Res. Commun. 43: 311, 1971.

Serial No.: NIEHS-PT-015
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Lung Conjugation Pathways and the Effects of Environmental Contaminants on the Enzymes Involved"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D. and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., R. T. Drew, Ph.D., C. Williams, M.S., and R. Easterling

COOPERATING UNITS: Synthetic and Analytical Chemistry Branch

MAN YEARS:

Total:	0.6
Professional:	0.3
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The biochemical mechanisms by which mammalian lung conjugates foreign compounds such as alkyl halides, aryl halides, phenols, amines, and epoxides and clears the water soluble conjugates formed from this organ is of toxicological interest, especially for volatile agents which may be inhaled. The objectives of this investigation are (1) to characterize the microsomal and soluble conjugating enzymes of lung and (2) to determine the effects of various environmental chemicals or pollutants upon these enzymes.

METHODS EMPLOYED: Differential centrifugation, spectroscopic techniques, inhalation exposures, and DEAE-cellulose chromatography of the rabbit liver and lung microsomal supernatant fractions.

MAJOR FINDINGS: Rabbit lung microsomes contain negligible glucuronyl transferase activity although liver microsomes from the same animals possess considerable activity. Studies of the soluble transferase enzymes were initiated with glutathione (GSH) S-aryltransferase since a direct spectrophotometric procedure for measuring the GSH conjugate is available. The time-activity, protein concentration-activity, cofactor concentration-activity, and pH-activity curves were found to be very similar for lung and liver enzymes. However, the specific activity of rabbit lung soluble fraction was only 25-30% that of liver (per mg soluble protein). DEAE-cellulose chromatography of liver and lung supernatants gave similar elution patterns of GSH S-aryltransferase activity. Preliminary

experiments have shown that rabbit lung has significant GSH S-epoxide transferase and GSH S-aralkyltransferase activity. Pretreatment of rabbits with hexachlorophene or aluminum chlorhydrate (by inhalation) did not cause any significant change in the GSH S-aryltransferase activity of liver or lung.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The conjugation of xenobiotics and xenobiotic metabolites by the lung may play a significant role in the overall toxicity of agents that enter the body via the pulmonary system. For example, the lack of detectable glucuronyl transferase activity in rabbit lung suggests, that in the absence of alternative conjugation systems such as sulphotransferase, toxic phenols may accumulate and cause localized damage in the lungs. The fate of any epoxides formed via pulmonary microsomal mixed function oxidation of aromatic hydrocarbon derivatives is also of considerable interest. In particular, the relative amounts of GSH S-epoxide-transferase and epoxide hydrase in lung are of concern since significant levels of reactive epoxides in this organ may be expected to cause localized tissue damage similar to the centrolobular hepatotoxicity observed after bromobenzene administration in the rat.

PROPOSED COURSE: Characterization of lung GSH S-aryltransferase and GSH S-epoxidetransferase activities will be completed and the in vivo and in vitro effect of environmental contaminants on the enzymatic activity determined. Other enzymes, including sulphotransferase, N-acetyltransferase and epoxide hydrase will also be investigated in lung.

Serial No.: NIEHS-PT-016
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Microsomal Mixed-Function Oxidase System from Rabbit Lung and Liver: Solubilization, Isolation, Reconstitution, and Specificity"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: T. C. Orton, Ph.D. and R. Simmons, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.1
Professional:	1.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: It is presently recognized that the microsomal mixed-function oxidase system of mammals exhibits species, sex, and tissue variations. In addition, the specificity of the system(s) can be altered by subjecting animals to various chemical treatments. A detailed accounting of the molecular processes responsible for the noted differences depends on the successful isolation and reconstitution of the various components of the mixed-function oxidase system. The primary objectives of this project with respect to the above are:

1. Solubilize the cytochrome P-450, TPNH cytochrome c reductase, and lipid fractions of the MFO system from rabbit liver and lung.

2. Reconstitute MFO activity from isolated components.

3. Compare substrate specificities for the liver and lung purified and reconstituted systems.

4. Compare the effect of inducer and inhibitor compounds on the liver and lung systems before and after purification and reconstitution.

5. Cross-match the components of the systems for determination of the fractions responsible for any variations that are encountered.

METHODS EMPLOYED: Cytochrome P-450 is solubilized via sonication and detergent treatment and fractionated out with ammonium sulfate precipitation. The reductase is obtained from acetone extracted microsomes. Lipid fractions are eluted from a DEAE cellulose column onto which solubilized microsomes have been placed. Most drug substrate metabolisms and bindings are assayed by standard procedures with the exception of testosterone metabolism which is being analyzed via T.L.C. utilizing newly developed solvent systems for the separation of the testosteronce metabolites.

MAJOR FINDINGS: The mixed-function oxidase system from untreated rabbit liver microsomes can be resolved into three soluble fractions and activity can be restored by recombining these fractions. The rate of metabolism of benzphetamine in the reconstituted system is the same as the rate found in untreated microsomes on a per n mole of cytochrome P-450 basis. Phenobarbital-induced cytochrome P-450 and 3-MC-induced cytochrome P-"448" can be solubilized from rabbit liver by the methods of Lu. Some differences have been found in the ammonium sulfate fractionation of these cytochromes as compared to those from the rat and the yield from rabbit is higher. A system for the separation of the 2β, 6β, 7α, and 16α hydroxylated products of testosterone by T.L.C. has been developed. The metabolism of testosterone by rabbit liver microsomes is presently being characterized with respect to protein and substrate concentrations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of mixed-function oxidase systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environmental pollutants, and pesticide detoxification are to be fully comprehended. This requirement makes it essential that investigations be carried out under conditions which allow the obligatory components of the system to be independently manipulated. With isolated, solubilized mixed-function oxidase systems the questions of specificity, mechanisms and kinetics can be approached in a more precise manner than that afforded through the use of microsomal preparations.

PROPOSED COURSE: The present work involving the solubilization and reconstitution of the MFO system from rabbit lung and liver will be continued. These systems will be characterized with respect to substrate specificity and the mechanisms which control specificity. The cytochrome fractions will be further characterized in an effort to distinguish the molecular basis for their differences. Experiments dealing with P-450 reductase, O_2-TPNH-substrate stoichiometry, cytochrome P-450-inhibitor complexes, and hydrogen peroxide production are also planned.

Serial No.: NIEHS-PT-017
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Chlorinated Environmental Contaminants: Sites of Storage and Effects on Drug Metabolism and Conjugating Systems"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., R. S. Chhabra, Ph.D., R. P. DiAugustine, Ph.D., T. E. Eling, Ph.D., G. E. R. Hook, Ph.D., and J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: R. Easterling, S. Gipson, B.S., and C. Williams, M.S.

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch and Animal Science and Technology Branch

MAN YEARS:

Total:	0.3
Professional:	0.2
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: Polychlorinated biphenyls (PCB's) and highly chlorinated insecticides, such as DDT, are found widely distributed in our environment. The objectives of this project are (1) to study the pharmacodynamics and the site of storage of some individual PCB isomers in liver and lung, and (2) to determine the inductive or inhibitory effects of this PCB pretreatement on lung and liver drug metabolizing and conjugating enzymes.

METHODS EMPLOYED: After homogenization and centrifugation of liver the hepatocyte lipid droplets, which occur randomly in vivo, are isolated as the "floating fat" layer. This "floating fat" layer and whole homogenates of control and treated animals are extracted and the PCB isomers present are quantitated by electron capture GLC. Drug metabolism activities are measured by standard spectroscopic techniques.

MAJOR FINDINGS: Both DDT and 2,4,2',4'-tetrachlorobiphenyl appear to be concentrated in the lipid droplets of hepatocytes. Oral administration of a single dose of 2,4,2',4'-tetrachlorobiphenyl (25 mg/kg) caused a significant increase in cytochrome P-450 content, biphenyl hydroxylase activity and benzphetamine demethylase activity of rat liver. Benzpyrene hydroxylase activity, on the other hand, did not change.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PCB derivatives and DDT and its analogs occur at detectable levels throughout most of the environment. The mammalian toxicology of PCB's has not yet been thoroughly evaluated, especially with regard to interactions with other chemicals and drugs.

PROPOSED COURSE: The effects of PCB administration (dose, route, etc.) upon the amount of compound localized in the lipid droplets, the time course for appearance and disappearance of the PCB in these lipids after a single dose, and the effect of starvation upon storage and release will be studied. The importance of the degree of chlorination on this process will also be ascertained. Results in mammals will be compared with those in aquatic species.

Serial No.: NIEHS-PT-018
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Hepatic Lipid Droplets. Isolation, Morphology, and Composition"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: Joan-Marie Schaefer and J. R. Fouts, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.7
Professional:	0.2
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: To study the morphology and composition of the floating lipid-rich fraction observed in rat liver homogenates following centrifugation at 9000-15,000X g for 10-20 mins.

METHODS EMPLOYED: Column, gas-liquid and thin-layer chromatography and electron microscopy

MAJOR FINDINGS: The floating lipid layer (FLL) was chiefly composed of triglycerides and cholesterol esters. Small amounts of free cholesterol, diglycerides, phospholipid, and protein were also present. No free fatty acids were detected. The FLL-triglyceride and cholesterol ester fraction consisted mostly of esters of linoleic acid, oleic acid, and palmitic acid. Electron micrographs of the FLL revealed numerous spherical osmiophilic droplets having a mean diameter of 1-2 microns surrounded by a very thin, dense, outer coat. Similar structures were observed as organelles in micrographs of the intact liver cell. The FLL-triglyceride decreased in rats starved 72 hours, but pellet triglyceride (homogenate minus FLL) was unchanged. Thus the FLL appears to be representative of the lipid-rich organelles observed *in vivo*, which probably function as a depot form of hepatic neutral lipid.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been previously shown that a large number of lipophilic drugs or compounds present in the environment, such as chlorinated hydrocarbons, are stored

preferentially in fatty tissues, such as omental fat, of mammals. The presence of appreciable amounts of aggregated neutral lipid in the liver would appear to influence the disposition and metabolism of lipophilic compounds in this organ, which is considered to be the major site for the metabolism of xenobiotics.

PROPOSED COURSE: These findings shall be enjoined with the efforts of others in the Pharmacology and Toxicology Branch who are investigating the uptake and distribution of various chlorinated hydrocarbons (including DDT and PCB's) in the mammalian liver - i.e. - are these pollutants localized in hepatic lipid droplets *in vivo*?

PUBLICATIONS

DiAugustine, R. P., Schaefer, J., and Fouts, J.R.: Hepatic Lipid Droplets. Isolation, Morphology, and Composition. *Biochemical Journal*, in press.

Serial No.: NIEHS-PT-019
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Studies of Mechanisms for Accumulation of Drugs and Pollutants in the Lung: Development of Isolated Perfused Lung and Investigation of Transport of Substances in Lung from Pulmonary Circulation and Inhaled Air"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: T. E. Eling, Ph.D., T. C. Orton, Ph.D., and M. Anderson, Ph.D. (Biometry Branch)

OTHER INVESTIGATORS: Douglas Parkes, B.S.

COOPERATING UNITS: Biometry Branch and Pathologic Physiology Branch (Radioautography and electron microscopy by Fred Talley)

MAN YEARS:

Total:	1.8
Professional:	1.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The principal objective of this project is to develop an isolated perfused lung preparation to study respiratory and non-respiratory functions, and their inter-relationships following exposure to pollutants via the airways and the circulation. Work has concentrated on the accumulative and metabolic processes for both xenobiotics and endogenous substances. Accumulation could give rise to toxic effects to lung or could serve as a detoxification mechanism.

METHODS EMPLOYED: Rabbits are anesthesized with pentobarbital. The blood is removed by cardiac puncture and heparinized. Lungs are excised from the animal. The trachea, pulmonary artery and vein are cannulated. The isolated lung is then connected to the perfusion apparatus. The lung is perfused with whole blood at a constant pressure via the pulmonary artery. The lung is inflated by a negative pressure which mimics normal inhalation and exhalation. ^{14}C and ^{3}H labeled drugs, pollutants, etc., are added to the perfusing blood and disappearance from the circulation is followed. The materials are extracted by a specific extraction procedure and the activity monitored by liquid scintillation. Metabolites of the compound in the blood and lung tissue are recovered by thin layer and paper chromatographic techniques. Viability of the perfused lung is confirmed by light and electron microscopy. Radioautography

is employed to locate the accumulated material in the lung.

MAJOR FINDINGS: Development of a successful isolated perfused lung preparation required solving the problem of perfusion at adequate blood flow rates, i.e., soon after isolation, lungs appeared to become congested and blood flow decreased precipitously. This difficulty was traced to stress produced in the rabbit during initial handling. In recent experiments, measurement of tidal volume, blood flow rate, gross edema (lung weight before minus lung weight after perfusion) and blood pH were within normal limits. Light microscopy indicated that the tissue was intact, not degenerated, and not edematous. Electron microscopy studies are not fully completed.

Studies with a number of amines with varying pKa's indicated that accumulation in the lung was often dependent on this pKa. Thus amines with high pKa's (e.g., amphetamine and imipramine) accumulate to a larger extent than amines with low pKa's (e.g., triazine herbicides). Preliminary radioautographs indicated greatest accumulation in the septal regions of the lung. The compounds localized to a lesser extent in the connective tissue of the bronchi and blood vessels. The material appeared to be located inside the cells.

Kinetic studies indicated that the transport from outside to the inside of the cells is facilitated by a carrier system. There appeared to be binding sites for these amines inside the cells. As further evidence for the storage capacity of the lung for these amines, accumulated compounds can be displaced from the lung into the circulation by a second amine.

Only one of the compounds studied (amphetamine) appeared to be metabolized to any appreciable extent (25%) in 60 minutes of perfusion. With the other compounds, less than 3% of the total added drug was metabolized by the isolated perfused lung.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance of this project can be divided into distinct areas:

1. Development of a method for the perfusion of an isolated perfused lung - this allows studies with the lung uncomplicated by other organ functions. This preparation is significant in the fact that it is perfused and ventilated similarly to an intact organ and thus correlative *in vivo* and *in vitro* studies are more likely.

2. The isolated perfused lung affords a good procedure to examine more directly than *in vivo* the effect of various pollutants and drugs on lung functions. Physiological and biochemical parameters can be examined and correlated.

3. The lung can act as an important site for accumulation of xenobiotics which could result in detoxification or altered toxicity. Thus the toxicity of a compound for the lung may be related to its ability to accumulate in the lung, for example paraquat. In addition, those xenobiotics which accumulate in the lung could effect the accumulation and storage of endogenous substances such as noradrenalin and 5-hydroxytryptamine. The elucidation of the mechanism of accumulation could afford a means for predicting possible toxic effects.

PROPOSED COURSE:

1. Further characterize the isolated perfused lung. Compare compliance, blood flow, and airway resistance of lung from normal and pre-exposed/treated animals.

2. Investigate in more detail the uptake and storage of amines. Is the process active or passive? What structure-activity relationships exist? What interactions occur?

3. Examine the accumulation of other classes of foreign compounds and drugs, e.g., acids, polycyclic hydrocarbons. Study interactions affecting uptake and release of stored chemicals by the lung.

4. Investigate the processes by which inhaled compounds enter the circulation in the perfused lung.

Serial No.: NIEHS-PT-020
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Metabolism of Sulfur Dioxide: Characterization and Modification of the Metabolic Pathway"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. J. Cohen, M.D., Ph.D.

OTHER INVESTIGATORS: K. V. Rajagopalan, Department of Biochemistry, Duke University, R. T. Drew, Ph.D., and F. Harrington

COOPERATING UNITS: None

MAN YEARS:

Total:	1.5
Professional:	1.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: This is an effort to understand one of the mechanisms by which the body metabolizes and detoxifies one of the most common air pollutants, sulfur dioxide. Studies are devoted to identifying, localizing, purifying, and characterizing the enzyme sulfite oxidase which oxidizes the hydrated form of sulfur dioxide to sulfate which can then be excreted in the urine.

METHODS EMPLOYED: Enzyme localization performed by differential centrifugation and by the use of digitonin. The enzyme is assayed spectrophotometrically using cytochrome c or ferricyanide as an electron acceptor; or polarographically using oxygen as an electron acceptor. "Structural" studies on the enzyme are performed using visual spectrophotometry and in conjunction with Dr. K. V. Rajagopalan using electron paramagnetic spectroscopy. Histochemical staining for activity utilizing redox specific dyes has also been studied.

MAJOR FINDINGS: In the rat, liver and kidney appear to be the major sources of sulfite oxidase with the lung containing about 2 percent of the liver activity. Study of developmental patterns from uterine to adult life has shown a concomitant increase in hepatic and pulmonary enzymatic levels occurring from birth to 3 weeks of age. Sulfite oxidase has been purified and characterized as to its prosthetic groups and found to contain a b_5-like cytochrome and molybdenum. The enzyme has been localized to the mitochondrial inter-membranous space by both enzymatic activity and spectral properties of the prosthetic groups.

Electron paramagnetic spectroscopy has enabled us to examine the state of the molybdenum in intact mitochondria at various pH values and to determine the effects of various reductants. We have found that rats exposed to levels of sulfur dioxide far greater than found in the atmosphere (50 PPM) showed no increase in the levels of the enzyme in either liver or lung. Attempts at localizing the enzyme by histochemical methods have led to the development of a sensitive assay for enzymes which produce hydrogen peroxide. The assay can be used for acrylamide gel staining.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Sulfur dioxide has been implicated in the pathogenesis of chronic respiratory disease. As a by-product of industry and fuel consumption it represents a serious potential hazard. An understanding of the metabolism and detoxification of this substance by the body and the effects of modifiers of this metabolism may allow alteration of the sensitivity of the body to this noxious agent.

PROPOSED COURSE:

1. Investigate the mechanism of oxidation of sulfite and relate it to mitochondrial electron transport and oxidative phosphorylation.

2. Determine what nutritional and/or environmental factors can lead to a modification of the enzyme activity *in vivo* and relate this to changes in the acute or chronic toxicity of sulfur dioxide.

3. Continue searching for a histochemical stain for sulfite oxidase activity.

4. Study the possible interaction of sulfur dioxide and other pollutants to produce enhanced effects on this enzyme system.

Serial No.: NIEHS-PT-021
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Lung Membrane-bound Nicotinamide Adenine Dinucleotide Glycohydrolase"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: S. Gipson, B.S., J. Abron, and R. T. Drew, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.7
Professional:	0.2
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Nicotinamide adenine dinucleotide glycohydrolase (EC 3.22.5), or NADase, which cleaves nicotinamide from NAD at the nicotinamide ribose link is very active in mammalian lung tissue and has been shown to greatly increase in lungs infected with M. tuberculosis. The mechanism of action of isoniazid is considered to be related to the formation of the isoniazid analog of NAD, which is catalyzed by NADase. The objective of the proposed studies are to study the properties and subcellular distributions of lung NADase and to investigate NADase activity in animals exposed to various environmental agents.

METHODS EMPLOYED: UV spectroscopy, pH-stat and paper chromatography were employed.

MAJOR FINDINGS: Lung NADase occurs exclusively in membrane fractions of the rat lung cell with highest activities in the 25,000 g and 105,000 g sediments. No NADase activity could be found in isolated rabbit alveolar macrophages. The membrane-bound enzyme exhibits a broad pH optimum between 5.9 and 6.9. The enzyme is poorly inhibited by isoniazid, but appears to readily form the isoniazid analog of NAD. So far only nicotinamide was able to exhibit a potent inhibition of NADase activity and may constitute an end product-inhibition system for the enzyme in vivo. Rats exposed to 100 ppm SO_2 for four hours/day for four days showed no significant change in lung NADase activity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been postulated by other workers that the delocalization of lung NADase might initiate cellular degenerative processes. A study of this enzyme and how it reacts to various environmental agents acting as lung irritants would allow a better understanding of the interdependence of lung NADase levels or distribution with tissue damage. An investigation of lung tissue levels and extent of retention of the isoniazid analog of NAD would help clarify the role of this compound as a tuberculostatic drug or in the initiation of the untoward effects observed in isoniazid treatment. An isoniazid pyridine nucleotide analog may inactivate vital cellular dehydrogenases.

PROPOSED COURSE: 1) To investigate further the activity of lung NADase in animals exposed to lung irritants. 2) To examine lung levels of the isoniazid analog of NAD and to establish whether the formation of this compound is dose-dependent and the extent of retention of the analog in the lung.

Serial No.: NIEHS-PT-022
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Prostaglandin Biosynthesis and Metabolism by Lungs. What are the Factors Controlling their Biosynthesis and Metabolism and What is Their Relationship to Physiological Function. Effect of Pollutants on This System"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: T. E. Eling, Ph.D.

OTHER INVESTIGATOR: D. G. Parkes, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: This project is directed toward obtaining a better understanding of lung function. The primary objective is to investigate the biosynthesis and metabolism of prostaglandins by the lung and to relate changes in steady-state concentrations of the various prostaglandins to biological effects. In addition, the effects of various environmental agents (SO_2, O_3, pesticides, etc.) on the ability of the lung to synthesize and degrade prostaglandins will be investigated. An attempt will be made to correlate the biological effect of pollutants and modulation of prostaglandin levels.

METHODS EMPLOYED: Prostaglandins are analyzed by a combination of quantitative thin layer chromatography and liquid scintillation counting. The radioactive prostaglandins, produced by lung tissue from the precursor ^{14}C-arachidonic acid, or the non-metabolized H^3-prostaglandins are extracted from the incubation medium, and separated on thin layer plates. The radioactive areas are measured by liquid scintillation techniques. Guinea pig lung serves as an enzyme and co-factor source for both biosynthesis and metabolism. Further identification of the prostaglandins uses a combination of thin layer chromatography, continuous gradient elution column chromatography, and chemical derivatization followed by gas chromatography and mass spectrometry.

MAJOR FINDINGS: The majority of prostaglandin biosynthetic studies have been conducted using the seminal vesicles of various animal species. The only study with lung tissue used crude homogenates of guinea pig's lungs. Our initial experiments established techniques for the separation and quantitation of prostaglandins. Using these techniques a qualitative study of the types of prostaglandins produced from the precursor ^{14}C-arachidonic acid showed the formation of $PGF_{2\alpha}$, small amounts of PGE_2 and an unknown prostaglandin. The unknown has been tentatively identified as 11-dehydro PGE_2 (PGD_2). Studies are currently being conducted to firmly establish the identity of PGD_2. PGD_2 formation has not been previously reported in lung tissue. A comparison of bovine seminal vesicles with guinea pig lung showed PGD_2 formed in small amounts by seminal vesicles but in large amounts by guinea pig lungs.

Prostaglandin synthetase activity in lung was found to be primarily located in the microsomal fraction with some activity in the mitochondrial fraction. Metabolic activity was located in the soluble fraction with PGE_2 acting as a better substrate than $PGF_{2\alpha}$. Addition of nicotinamide to the incubation medium enhanced the metabolism of PGE_2 and $PGF_{2\alpha}$ by soluble fraction but did not alter the biosynthetic activity of microsomal fraction of lungs.

The nature of lung prostaglandin synthetase was investigated. The enzyme assay was optimized with respect to protein concentrations, incubation times, pH, substrate and cofactor concentrations. A cofactor and a (protein) inhibitor were found in lung soluble fraction. The nature of the cofactor and the inhibitor are currently being investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Prostaglandins represent a new class of endogenous substance which possess unique biological activity in lung. Prostaglandins of the E class are potent bronchial dilators, while prostaglandins of the F class are potent constrictors. Both classes of prostaglandins have been implicated in inflammatory responses. Since these substances are not stored in most tissue, control of biosynthesis and metabolism is important to their physiological activity. Pathological changes or conditions produced in various disease states or in response to pollutants or environmental agents could involve an effect on either prostaglandin biosynthesis or metabolism. Understanding the mechanisms for the control of prostaglandin levels in lung tissue should contribute to the overall understanding of biological functions.

PROPOSED COURSE:

1. Further studies will be conducted to more firmly establish the identity of the prostaglandins formed by lung tissue.

2. Investigate the cofactors that control the rate of biosynthesis and nature or type of prostaglandins formed in lung. What is the nature of the cofactors and the inhibitor found in the soluble fraction of lung tissue?

3. Effect of pollutants on prostaglandin biosynthesis and metabolism will be studied *in vivo* and *in vitro*.

4. Examine the effect of the various prostaglandin produced by lung tissue on lung physiological and biochemical functions.

Serial No.: NIEHS-PT-023
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Inhalation Toxicity of Compounds Common to Household Aerosols"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. T. Drew, Ph.D., J. R. Bend, Ph.D., and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: R. P. Roth, Ph.D., E. Lores, B.S., M. Riley, J. Schroeder, and F. Harrington

COOPERATING UNITS: Pathologic Physiology Branch (Histology) and Animal Science and Technology Branch (Pathology)

MAN YEARS:

Total:	0.7
Professional:	0.3
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are to determine the acute and chronic inhalation toxicity of selected compounds common to household aerosols and to identify any potential synergistic interactions.

METHODS EMPLOYED: Three different materials are currently being investigated, hexachlorophene, Rehydrol ASC (the aluminum hydroxychloride-ethylene glycol complex used as the antiperspirant in most aerosol underarm deodorants), and "Airwick."

Aerosols of hexachlorophene or Rehydrol ASC are generated from an aqueous suspension or solution, respectively, using a compressed air-driven nebulizer. Rats and rabbits have been exposed to hexachlorophene and Rehydrol ASC for 4 hours/day for periods of up to 5 weeks in appropriate exposure chambers. Rabbits have been exposed to Airwick vapors by passing the chamber intake air through a box having a large surface area saturated with liquid Airwick. The effects of exposure to these compounds are being assessed by histological techniques and by investigating the mixed function oxidase (MFO) activities of both lung and liver microsomes after exposures.

MAJOR FINDINGS: a) Hexachlorophene: Histological examination of lungs and livers from rats exposed for 5 weeks (4 hours/day, 5 days/week) showed no effects of the exposure. Single exposures or 5 consecutive daily exposures had no effect on MFO activity in rabbit liver microsomes and slight to variably stimulating effects on lung microsomal MFO activity.

b) Rehydrol ASC: Four of ten rats survived after 8 exposures to Rehydrol ASC. Two were sacrificed after the last exposure and two were held for 3 weeks and then sacrificed. These two rats developed a fibrosis at the level of the respiratory bronchioles. A survey of the MFO activities in rabbit lung has shown that the lung mass increases, the microsomal yield in mg protein/gm lung increases and the MFO activities decrease.

c) Airwick: In a preliminary experiment rabbits received single or multiple exposures to Airwick vapors. Twelve hours after the last exposure the animals were sacrificed and the MFO activities of both lung and liver microsomes were measured. There were no statistically significant differences between the control and exposed groups.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of the aerosol "spray can" dispenser has resulted in a very complex and wide variety of materials being available in respirable form. While the acute toxicity of many of the "active ingredients" in such dispensers has been investigated, little is known about the chronic toxicity of these compounds or the toxicity of combinations of these compounds and "inert ingredients" of the pressurized cans. Elucidation of the chronic toxicity of these compounds and their potential combinations can be of vital importance to environmental health. The lung is the first line of defense when these compounds are inhaled and a knowledge of the metabolic transformations which occur in the lung is necessary to assess some of the indirect effects of exposure to these compounds.

PROPOSED COURSE: This project will be continued, with added emphasis on the etiology of the histological lesions, initiation of chronic studies, and investigation of other compounds and mixtures.

Serial No.: NIEHS-PT-024
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Inhalation Toxicity of Pesticides and Combinations of Pesticides"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. T. Drew, Ph.D.

OTHER INVESTIGATORS: Johnny Block, Ph.D., J. R. Fouts, Ph.D., J. Schroeder, E. Lores, B.S., M. Riley, and F. Harrington

COOPERATING UNITS: None

MAN YEARS:

Total:	0.7
Professional:	0.4
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The object of this study is to determine the toxic effects of acute (single) and chronic inhalation exposures to single pesticides and to combinations of pesticides and/or other airborn pollutants.

METHODS EMPLOYED: The initial phase of this project has been to identify potential compounds for investigation. Compounds have been selected on the basis of chemical classification; use, both recent past use and predictions of use over the next five years; and likelihood of being inhaled. Some of the compounds selected for preliminary investigation include Maneb, Zineb, Dichlorvos, Carbaryl, Toxaphene, and chlordane.

Studies designed to demonstrate if these compounds might cause synergistic effects have begun. The length of time a rat sleeps after an IP injection of hexobarbital can be used as one index of the mixed function oxidase activity of liver microsomes. Hexobarbital sleeping time (HST) experiments have been carried out in rats after intragastric intubation of Maneb, IP injection of Carbaryl and IP injection of Dichlorvos.

MAJOR FINDINGS: Twenty four hours after a single dose of Maneb, HST is increased indicating a decrease in MFO activity. The same is true 2-4 hours after a single IP injection of Carbaryl. Dichlorvos injections seemed to have no affect on HST. When both Maneb and Carbaryl are given, HST is increased

over each compound given alone, but the effect appears additive, not synergistic.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development and use of pesticides have caused environmental contamination by a wide variety of compounds. The acute toxicity (oral and dermal) of these compounds, for the most part, has been investigated. In some cases, the acute inhalation toxicity has also been studied. However, toxicity due to combinations of pesticides entering the body by different routes has received little attention. In addition, other compounds such as gaseous air pollutants or even alcohol may react synergistically with one or more pesticides. Elucidation of the chronic inhalation toxicity of selected compounds, and identification of possibly synergistic combinations of pesticides, pollutants and drugs are long term goals of this project.

PROPOSED COURSE: This study will continue with added emphasis on inhalation studies of selected combinations of pesticides and/or chemicals as equipment and personnel become available.

Serial No.: NIEHS-PT-025
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Survival and Development of Mammalian Embryos after In Vitro Exposure to Selected Chemicals and Conditions"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. S. Elliott, Ph.D. and R. R. Maurer, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D., C. D'Onofrio, B.S., R. Kellam, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	3.75
Professional:	2.00
Other:	1.75

PROJECT DESCRIPTION

OBJECTIVES: This is an effort to determine the effects of various environmental alterations, alone and in combination, upon application to mammalian gametes and early embryos in vitro. Existing methods used to culture mammalian embryos vary among laboratories and most investigators have not evaluated surviving fetuses from cultured embryos for the presence of congenital alterations. Therefore, the first objective will be to establish a repeatable method, in this laboratory, for the culture of early cleavage stage embryos of several species to the blastocyst stage; to establish reliable methodology for the transfer of mammalian embryos into suitable recipient females; to determine whether the cultured blastocysts are viable and capable of "normal" development; and, to use this system to test specific chemicals or conditions for their potential to alter subsequent development or survival.

METHODS EMPLOYED: Embryos are recovered by conventional methods from females on Days 1, 2, 3 or 4 of gestation depending upon the cell stage desired. The embryos are cultured in vitro for periods of time ranging from one to three days or until they reach the blastocyst stage. Initially, the culture conditions (e.g. medium, air flow, etc.) are varied until from 70 to 80% of the embryos reach the blastocyst stage during a time course that approximates that seen for in vivo development. Cultured and noncultured blastocysts are transferred to pregnant, pseudopregnant and hormone-induced females to determine the optimal recipient with regard to viability and embryonic development of the transferred embryos.

The recipients are killed one or two days before term, the reproductive status of each is recorded and the fetuses are examined for the presence of structural alteration (gross, visceral, and skeletal).

MAJOR FINDINGS: This program has just started. Mouse, rat, and rabbit embryos have been successfully cultured for one to three days. Information obtained thus far suggests that the rate and ultimate stage of development *in vitro* is dependent not only upon the biochemical parameters, but also upon physical parameters inherent to the culture system. Other preliminary results indicate that strains of mice differ in maternal capability under the given restrictions.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system permitting culture of mammalian embryos with subsequent transfer to suitable recipient females would facilitate evaluation of "normal" developmental parameters and would aid in distinguishing direct from indirect effects of environmental insults on embryo development. The information obtained would increase our understanding of embryonic requirements and could specify the sequential steps most susceptible to environmental insult.

PROPOSED COURSE: We will continue to study the effects of culture upon subsequent development of the embryos after transfer to appropriate recipients.

Serial No.: NIEHS-PT-026
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Storage of Mammalian Gametes and Embryos"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. S. Elliott, Ph.D., R. R. Maurer, Ph.D., L. A. Moustafa, Ph.D., and R. E. Staples, Ph.D.

OTHER INVESTIGATORS: J. Putnam, B.S., C. D'Onofrio, B.S., R. Kellam, B.S. and C. Sloan, M.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: This is an effort to establish methodology for the storage of viable mammalian gametes and embryos and to investigate the effects of reversible cleavage inhibition. There is a report in the literature that a high incidence of survival to term was obtained after transfer of previously frozen (-79°C) four-day mouse blastocysts to recipient female mice. The first objective is to verify this method and to assess the effects of freezing on subsequent development. The effects that freezing has upon the fertilizing capacity of rabbit spermatozoa and subsequent embryonic and fetal development will also be assessed.

METHODS EMPLOYED: Embryos are recovered by conventional methods from female (CF1-S strain) mice on Day 4 of gestation. The embryos are frozen and stored at -79°C for periods ranging from 15 to 75 minutes, thawed, and transferred to recipient females of the same strain. Fetuses are removed by caesarean section one or two days before the time of normal delivery and examined for teratological changes. Rabbit spermatozoa are collected, extended, frozen and stored at -196°C for various periods of time. Upon thawing the semen is used to inseminate suitable females and subsequent embryonic and fetal development is assessed.

MAJOR FINDINGS: This program has just been initiated. The originally reported freezing technique used for blastocysts, and several modifications, have been studied with little success. The published results have not been confirmed. Rabbit spermatozoa, however, can be frozen with retention of fertilizing ability upon thawing.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One effect that various environmental factors have on the mammalian embryo is to inhibit cleavage. It is therefore more than passing interest to study the mechanisms of cleavage inhibition and its effect upon subsequent embryo survival and development. The establishment of a freezing method for storage of mammalian eggs or blastocysts would greatly facilitate experimentation by providing a ready source of embryos. The ability to successfully freeze spermatozoa would permit more extensive use of selected males, thereby limiting genetic variation among experimental groups.

PROPOSED COURSE: Since these studies have only recently been undertaken they will be pursued as outlined.

Serial No.: NIEHS-PT-027
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effect of Direct Application of Test Agents to the Mammalian Embryo on Subsequent Viability and Development"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Laila A. Moustafa, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D. and C. Sloan, M.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	2.6
Professional:	1.1
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: The predictability for the human of information gained during routine teratological studies has been minimal, largely due to wide differences in teratological response from species-to-species in test animals. With the thought that intraspecies variability of embryonic response to applied chemicals might be only a fraction of that observed after administration of a test agent to the mother (pregnant animal), the major objective of this study is to bypass the maternal influence on the distribution and metabolism of an agent, and to thereby investigate the susceptibility of mammalian embryos to test agent by direct application while in utero or by direct injection while in vitro.

METHODS EMPLOYED: In addition to conventional intra-uterine injections, a micrurgical technique recently developed by the principal investigator has been adapted for direct administration of agents into mammalian blastocysts in vitro. After treatment, blastocysts are either transferred to recipient females or prepared for study by autoradiographic, histochemical and cytological techniques.

MAJOR FINDINGS: An embryo culture system has been established and expertise has been developed in the technique of embryo transfer. These advancements ensure a substantial number of transferred embryos developing into viable fetuses. Intra-uterine deposition and local injection of thalidomide into mouse and rabbit blastocysts in vitro have been initiated to test the value

of these approaches. Single cells are also being successfully transplanted into preimplantation mouse blastocysts; and viable, chimaeric young are being obtained.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The microsurgical and micrurgical approaches permit study of the direct effect of various compounds on the developing mammalian embryos. These techniques provide a means of bypassing maternal variability in distribution and metabolism of test agents. It is hoped that "between species variability" in embryonic response will be minimized by these procedures, and if so, the results obtained may provide increased predictability with regard to what might be expected upon exposure of human embryo to a given concentration of the parent test agent.

Information on the occurrence and probable mechanisms of cellular reaction with chemicals may be provided by investigating some of the early cytological-biochemical changes in developing embryos after direct administration of known teratogens by micrurgical route or during culture.

PROPOSED COURSE: Cases of positive teratology will be pursued to determine whether the teratogenic activity of a compound parallels its relative ability to bind with embryonic tissue. Through the use of inbred strains it is possible to hold a gene pool constant to permit study of an altered environment; it is also possible to study any environmental agent in several gene pools or in chimaeric animals. Such studies would be employed at later phases in delineating the conditions affecting the teratogenic expression of an agent found to be teratogenic by routine screening or by use of micrurgical techniques.

Serial No.: NIEHS-PT-028
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "The Teratogenicity of Dimethyl Sulfoxide"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Staples, Ph.D.

OTHER INVESTIGATORS: J. Putnam, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.5
Professional:	0.1
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: Dimethyl sulfoxide (DMSO), a chemical solvent with therapeutic properties, was teratogenic in fetuses taken from their dams on Days 9 to 11 of gestation after administration to hamsters on Day 8 of gestation (Ferm, 1966). Since only embryo lethality was seen in the rat (Juma and Staples, 1967) at term after s.c. administration of DMSO, additional more thorough studies were conducted in both of these species and in the mouse (Staples and Pecharo, 1972). In the present study the susceptibility of the rabbit to DMSO is being tested to supply additional comparative information. This is also considered appropriate since the results presented in a previous report (Caujolle, 1967) were fragmentary and inconclusive, and since many techniques for the study of early embryo development are available for the rabbit.

METHODS EMPLOYED: Dutch Belted rabbits are administered DMSO subcutaneously between Days 8 and 12 of gestation. After sacrifice (Day 28 of gestation) the reproductive status of the dams is recorded and each fetus is weighed and examined for structural alterations (gross, visceral, and skeletal).

MAJOR FINDINGS: A pilot study indicated that subcutaneous doses of DMSO at 5.0 gm/kg/day is lethal to most does before the third day of dosing, but that dosages of 3.0 gm/kg/day for four days can be tolerated by most dams. The latter dose is being used to assess teratogenic potential in the rabbit.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Large doses of DMSO have been given to the human by the dermal route as therapy for muscular pain, arthritis, etc., and by various routes as a solvent for therapeutic agents. Its cryoprotective properties have also been used for preservation of cells and gametes. This compound therefore is of widespread biomedical interest. DMSO is also of interest as a teratogen since it has been shown to be teratogenic in the hamster and the mouse, but not in the rat; this species difference is difficult to explain with information available to date, particularly since DMSO readily penetrates the soft tissues of animals but is not metabolized to a great extent.

PROPOSED COURSE: Further studies will be conducted to determine the viability and normalcy of development of blastocysts of these species after DMSO application *in vitro* and locally to the uterine lumen. The doses required to alter the normal process of development after administration of DMSO to the dam and to the conceptus *in utero* and *in vitro* will be compared; differences in the spectrum of response will be delineated.

PUBLICATIONS

Staples, R. E. and Pecharo, M. M.: Species differences in DMSO teratology. *Teratology*. 5: , 1972 (Abstract) (in press).

OFFICE OF THE ASSOCIATE DIRECTOR FOR EPIDEMIOLOGY AND BIOMETRY

OFFICE OF ASSOCIATE DIRECTOR FOR EPIDEMIOLOGY AND BIOMETRY
Summary Statement

Program planning and development efforts in the epidemiology and biometry areas have been predicated on assessment of need for supporting services, availability of resources, and competing research priorities. The primary function of the Biometry Branch has been and will continue to be direct participation in the statistical design and analysis of laboratory experiments. In addition, the construction of mathematical models and development of analytic procedures essential to the evaluation of multifactor experiments of interacting environmental agents constitute statistical research areas of high priority and current effort. Biometry Branch personnel have contributed directly to a wide variety of technical activities in support of the Office of the Director, NIEHS, and other Federal agencies.

Personnel restrictions have served to limit development of an active intramural epidemiology program and an Epidemiology Branch has not been organized. Program development in epidemiology is restricted to contract research in areas of teratology, mutagenesis, and health effects of pesticides and heavy metals (see p. 3). Epidemiologic studies are developed in close cooperation with the intramural scientific staff.

BIOMETRY BRANCH

BIOMETRY BRANCH
Summary Statement

The primary function of the Biometry Branch is to provide statistical analyses of scientific research data for the intramural program of NIEHS. Because of experimental and biological variation, it is necessary to establish the precision of experimental observations and to ascertain whether the effects observed for potentially toxic agents are likely to be real reproducible effects or a result of chance variation. Also, with limited facilities and resources, evaluation of experimental protocol is necessary to determine the magnitude of effects which can be detected with confidence.

Considerable use is made of the computer facilities at the Triangle Universities Computation Center, Research Triangle Park, N. C., and those of the Division of Computer Research and Technology, NIH, Bethesda, Maryland. Besides utilizing the computers for statistical and mathematical analyses and research, other computing is performed for the scientific staff. In addition, training courses have been provided on the use of the WYLBUR system for administrative personnel.

Statistical analyses performed for the scientific staff of the NIEHS intramural research program are summarized in the following paragraphs.

PHARMACOLOGY AND TOXICOLOGY BRANCH

In order to study the uptake mechanism and the storage capacity of the lung for a series of compounds at various dose levels, two characteristics of the uptake vs time curves were examined, i.e., the initial velocity of uptake and the ratio of a tissue concentration to the blood concentration at steady state conditions. For several of the compounds, the uptake appeared to be carrier mediated. In addition, there seemed to be storage sites in the lung for these compounds. Further experiments are being performed to substantiate these preliminary findings. Mathematical models which will reproduce these time curves from some basic chemical parameters are being investigated.

A computer program is being developed to construct an isokinetic gradient for rate-zonal centrifugation from the approximate boundary and initial conditions. The effects of the assumption of an ideal particle (smooth and spherical) on the gradient profile is being investigated. A comparison between these theoretical investigations and experimental results will be performed.

Four methods of homogenizing rabbit lungs were studied experimentally by investigation of their subcellular fractions. A most suitable method was statistically determined as judged by marker enzyme concentrations and yields.

A study investigating the developmental aspects of hepatic and extrahepatic drug-metabolizing enzyme systems in the rabbit during the first month of life was analyzed. Because of the nature of the statistical treatment required, a general computer routine was written for the analysis of replicated experiments having unequal sampling and possible replicate effects and/or a replicate by treatment interaction.

Statistical analyses were performed to estimate the effects of carbaryl on acetylcholinesterase (ACH) activity in maternal and fetal rat liver. In spite of considerable experimental variation, it was possible to demonstrate depressions of ACH activity in maternal and fetal liver by carbaryl. An alteration of the sampling plan was suggested for future studies in order to obtain more precise results.

PATHOLOGIC PHYSIOLOGY BRANCH

Data were analyzed from a study investigating the effect of several doses of lead on rats being fed a low calcium diet. A number of variables were examined including weight gain, various blood values and lead content in the femur, kidney and urine. Maximum likelihood techniques were employed to estimate and place 90% confidence limits on the lead dosage that produced a response equivalent to that observed for 200 µg/ml lead given to a group of animals on a normal calcium diet.

Analyses were performed for a series of experiments investigating the effect of subcutaneous administration of mercury on rat urinary function. Treatment vs control comparisons were made for a number of measures related to urinary function such as serum creatinine, creatinine clearance and kidney weight. In a related series of experiments, the relative deposition of mercury within various components of the central nervous system was studied.

Statistical techniques were employed to determine whether or not female opossums examined during the first six months of 1971 showed evidence of seasonal variation of litter size and perinatal mortality. A regression analysis indicated no significant linear trend in litter size. However, there was a marked ($P < .05$) increase in perinatal mortality in late May and June. In a separate study the effect of U-18, 666A, triparanol and clofibrate on lipid levels in the brains of opossums were examined.

ANALYTICAL AND SYNTHETIC CHEMISTRY BRANCH

The electronic distribution in some polychlorinated biphenyls and other biphenyl derivatives is being investigated, as well as the energy surface as a function of the dihedral angle between the two rings. This information will be used to help interpret the NMR spectra of these compounds and to investigate the charge transfer complexing potential of the polychlorinated biphenyls. The all valence electron CNDO/2 procedure in molecular orbital theory is being used to perform the calculations.

An analysis was made of non-specific heme transfer which occurs during the preparation of microsomal particles from mammalian liver. Comparison of two hybrids resulted in no significant differences, but this may well have been

a reflection of the small sample sizes employed. Thus, tables of required sample sizes for varying percent differences, significance levels and power were constructed in order to help the investigator evaluate the relevance of the findings and facilitate the planning of future experiments.

ANIMAL SCIENCE AND TECHNOLOGY BRANCH

Background levels of certain variables in untreated Charles River mice sacrificed on days 13-18 of gestation were investigated. Curve fitting techniques were employed to determine the functional form of the increase in fetal weight over time. It was found that a quadratic model fit the data well, explaining 98.1% of the observed variability. Placental weight, maternal liver/body weight ratio and fetal mortality were also examined.

Nonparametric test procedures were used to determine the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on fetal mortality and anomalies in two strains of mice. In addition, treatment vs control differences in fetal and placental weight were tested by a 3-level nested classification analysis of variance with unequal sampling. Similar techniques were used in a study investigating the teratogenic potential of hycanthone methane sulfonate.

Data were analyzed from a study investigating blood levels in beagles at various time periods following inoculation with bacteria. Analysis of covariance procedures were performed to determine whether or not blood levels differed significantly from those of uninoculated control animals.

Statistical analyses were performed to determine the effect of cobalt sulfate on subsequent cobalt levels in the heart, liver, spleen, brain and blood of mice after three weeks of treatment. Analyses of variance techniques revealed significant differences among the three groups for the heart, liver and spleen. The addition of ethanol was found to have little effect.

Statistical techniques were employed to determine the effect of several metal salts on the spleen weight of mice inoculated with Rauscher leukemogenic virus. It was found that both the lead and cobalt treated animals showed significantly increased spleen weight relative to untreated controls. In a related metal salts study, Fisher's exact test for 2x2 tables and the Mantel-Haenszel procedure were employed to make treatment-control mortality comparisons.

An experiment was performed to investigate the effect of three levels of poly I/poly C and bovine serum albumin on serum protein levels in mice. Analysis of variance procedures were employed to test for treatment-effects. Significant differences for some serum proteins were found.

RESEARCH SERVICES BRANCH

Data were analyzed from a study investigating the effect of methylmercury on the cochlea of guinea pigs. Daily doses of 2 mg/kg MeHg were administered to animals for periods ranging from one to seven weeks. Certain areas of the cochlea showed a significantly increased proportion of abnormal cells when compared with untreated controls, but the extent of damage appeared to be unrelated to length of exposure.

EPIDEMIOLOGY

A study of airplane maintenance workers was performed to determine whether exposure to zinc chromate paint by inhalation results in an increased risk to lung cancer. Mortality follow-up through 1966 has been completed. Data on decedents' occupation, birth data, sex, race, exposure, age at death and cause of death have been prepared for computer input. Statistical analyses will be performed to determine the role, if any, of exposure to chromium by inhalation in disease mortality.

OFFICE OF THE DIRECTOR

The following projects were performed for the Director of NIEHS or staff members of the Office of the Director. Frequently, these projects entailed collaborative efforts with other government agencies.

A comprehensive statistical analysis of the teratogenic potential of nitrilotriacetic acid (NTA) was performed for experimental results obtained by Procter and Gamble, Bio-test Laboratories, International Research and Development Corporation and NIEHS. The experimental animal studied was the rat; treatments generally were administered orally. The addition of 40 mg/kg of NTA to 8 mg/kg of MeHgCl resulted in an increase in edema and cleft palates. When NTA was added to $CdCl_2$ with both administered subcutaneously, an increase in maternal and fetal mortality was observed. However, with oral administration, no deleterious effects were detected. No other consistent patterns of maternal toxicity or of fetal toxicity or teratogenesis were noted when NTA was added to cadmium or methylmercury exposure.

Members of the Biometry Branch collaborated with the National Cancer Institute in a statistical analysis of tumor data from a two-year rat feeding study of NTA conducted by the Procter and Gamble Company. The analysis revealed that female animals autopsied during the 21-23rd months showed a significant increase in non-mammary tumors. Similarly, for the visceral organ tumors, taken collectively, there was a statistically significant increase in the treated animals. Both the analysis and the interpretation of its results were complicated by the fact that a sizable proportion of the animals in the study were never autopsied.

The amount of information needed to identify subjects from a National Death Index was estimated by a simulation based on telephone directory data. The simulation indicated that for identification with a minimum of incorrect matching of death index entries to study group cases, the following information is needed when available from a death certificate: first name, middle name, last name (including maiden name), birth date (mo/day/yr) or age and state of birth. The last 4 digits of the social security number would be highly desirable in the absence of any of the preceding information.

The General Foods Corporation conducted two-year feeding experiments for the National Cancer Institute to assess the carcinogenic activity of piperonyl butoxide, 2,7FAA and DDT. Statistical analyses conducted at NIEHS indicated that both studies showed an increase in bile duct hyperplasia and benign liver neoplasms in females at the high dose of piperonyl butoxide. There was also an increased incidence of malignant tumors at the high dose of 2,7FAA.

An experiment was performed by the Hazelton Laboratories for the National Cancer Institute to determine the effects of inhalation of NiS, ozonized gasoline and cigarette smoke (each with and without fluorocarbon injection) on the incidence of lung cancer in rats. Lung tissue slides were re-examined at NIEHS by the Pathologic Physiology Branch. The fluorocarbon injection produced no detectable effect. The NiS group showed a statistically significant increase in the proportion of animals with lung cancer.

STATISTICAL AND MATHEMATICAL RESEARCH

The following statistical and mathematical research was motivated by a need for improved techniques for the analysis of biological research data.

Investigations of statistical procedures for analyzing teratological data were continued. A probabilistic model was developed to examine the possible relationship between the estimated proportions of abnormal and dead fetuses within a given litter. Computer simulations were used to evaluate the robustness of the t-test applied to data in which a large proportion of the animals was unaffected by either of the treatments being compared. Finally, the extent to which the proportion of dead, abnormal and normal fetuses depends on the number of implantations was determined for three different strains of (control) mice. Results of the above investigations were presented in invited talks given at the Symposium on Statistical Aspects of Pollution Problems (IASPS) and at the Central Regional Meetings of the Biometric Society and the American Statistical Association.

The development of new nonparametric procedures for application to survival data is being continued. A probabilistic model has been studied for describing the occurrences of particular diseases in a competing risk framework. Nonparametric techniques are used to estimate the model giving both the disease incidence and cumulative mortality adjusted for competing causes of death. A distribution-free method of estimating non-lethal disease incidences has also been investigated. Finally, methods for testing treatment interactions on general survival times have been developed.

Nonparametric (requiring no assumptions about the form of the distribution of the data) tests (1) for interaction in factorial experiments and (2) for testing equality of means in a completely randomized design against ordered alternatives when responses are censored are being developed.

Research into developing new or improved clinical trial designs has been continued. Of particular importance is a new procedure which does not assume an instantaneous response to the treatment. The response time is taken to be variable with possibly different distributions for the treatment groups. Play-the-winner sampling is used which has the property of decreasing the expected number of patients on the inferior treatment.

Using a series of sulfa drugs as a mode for structure-activity studies, calculations have been performed with the CNDO/2 procedure in molecular orbital theory. Correlations with the charge density in an atomic orbital of a nitrogen atom have been obtained. Also, correlations with the energy of the lowest empty molecular orbital have been determined.

Techniques were developed for performing tests of goodness-of-fit, using existing tables of critical values for the Kolmogoroff, Cramer-von Mises and chi-square statistics, in the case where the underlying distribution contains unknown parameters.

For complete factorial experiments, statistical techniques are available which provide best estimates or tests of effects. When some of the treatment combinations are missing, analyses become quite complicated. In order to simplify calculations, analytical modifications are available for experiments containing two factors. These modifications were extended to the general case of missing treatment combinations with more than two experimental factors.

OTHER PROFESSIONAL ACTIVITIES

Dr. D. W. Gaylor was appointed Chairman of the Statistics Sub-group for the Task Force to recommend experimental protocol and to indicate research objectives and problems for the newly formed National Center for Toxicological Research, Pine Bluff, Arkansas.

Dr. D. W. Gaylor served as Adjunct Associate Professor of Statistics, North Carolina State University, Raleigh, North Carolina. Activities included teaching a graduate course on Advanced Analysis of Variance and Components of Variance and serving on the Examination Committee for Ph.D. Candidates. Dr. Gaylor acted as advisor for a Ph.D. thesis entitled, "Estimating variance components for two-way disproportionate data by the method of unweighted means," by T. D. Hartwell. The method of unweighted means was extended to the case of two levels of nesting within cells. Also, techniques for estimating variance components were developed for the case of empty cells.

Dr. D. G. Hoel served as an Adjunct Assistant Professor of Statistics, Department of Biostatistics, University of North Carolina, Chapel Hill, North Carolina. His duties included teaching a section on sequential clinical trials for a special topics graduate course in Biostatistics.

PUBLICATIONS

Hartwell, T. D. and Gaylor, D. W.: Unweighted means for a two-way classification with nesting. Biometrics (In Press)

Hook, G. E. R., Bend, J. R., Hoel, D. G., Fouts, J. R. and Gram, T. E.: Preparation of lung microsomes and a comparison of the distribution of enzyme between subcellular fractions of rabbit lung and liver. J. Pharmacol. Exp. Ther. (In Press)

Serial No.: NIEHS-B-004
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Statistical Methodology and Modeling in the Analysis of Teratology Data"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: D. G. Hoel, Ph.D., J. K. Haseman, Ph.D., M. D. Hogan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	1.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To modify and adapt existing statistical techniques and to develop probability models and new procedures for the analysis of teratology data.

METHODS EMPLOYED: (1) A probabilistic model was developed to examine the possible relationship between the estimated proportions of abnormal and dead fetuses within a given litter. (2) Computer simulations were used to investigate the robustness of the t-test applied to data in which a large proportion of the animals was unaffected by either of the treatments being compared. (3) Large sets of experimental data from three different strains of control mice were reviewed to determine to what extent the proportion of dead, abnormal and normal fetuses in a litter depends on the number of implantations or live fetuses.

MAJOR FINDINGS: (1) A probability model of an animal litter indicated that it is possible to produce an increase (decrease) in the conditional probability of a fetus having an anomaly, given that it is born live, merely by decreasing (increasing) the probability of death for the litter. Thus, it would seem appropriate to make treatment vs control comparisons of the observed proportion of abnormal fetuses only where there is no change in mortality among the treated litters. (2) The t-test tends to be very conservative where a large proportion of the treated and control litters exhibit no effect; and the censored U-test, or its large sample approximation, should be used. Exact, small sample statis-

tical tables for the censored U-test have been constructed. (3) There was no consistent relationship between the proportion of dead, abnormal or normal fetuses and the number of implants or live fetuses, particularly when litters with extreme implant sizes were excluded from the analyses.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the laboratory animal often serves as a biological model for man in making decisions concerning the safety of existing or proposed agents, improved statistical procedures for analyzing laboratory data on teratology are needed.

PROPOSED COURSE: There are many remaining statistical problems in this area that should be explored. These include the adaptation or extension of a procedure such as Quade's nonparametric rank analysis of covariance to the problem of dependencies on either implantation size or live litter size; the statistical treatment of fetuses with multiple anomalies; and the comparison of anomalies according to their degree of severity.

PUBLICATIONS

Hoel, D. G.: Some statistical aspects of experiments for determining the teratogenic effects of chemicals. IASPS Symposium of Statistical Aspects of Pollution Problems. (In Press).

Serial No.: NIEHS-B-005
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Clinical Trials and Statistical Selection Procedures"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To develop efficient experimental designs for selecting the best of several experimental treatments.

METHODS EMPLOYED: Data from controlled laboratory or clinical trials are assumed to occur sequentially in time where the individual responses are dichotomous. Sequential regions have been developed for deciding when to terminate the experiment and which treatment to select as most effective.

MAJOR FINDINGS: New and improved procedures are continuing to be developed. Of particular importance is a new procedure which does not assume an instantaneous response to treatment and yet uses a play-the-winner treatment assignment design.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The emphasis of these sequential designs is to minimize the number of trials on inferior treatments. This has been overlooked in classical clinical trials and is a critical concern when dealing with human populations.

PROPOSED COURSE: Continued improvement in the efficient use of laboratory and clinical trial data is required. Also further investigations into delayed clinical responses is needed.

PUBLICATIONS

Hoel, D. G.: An inverse stopping rule for play-the-winner sampling. J. Amer. Statist. Assoc. 67: 148-151, 1972.

Hoel, D. G. and Sobel, M.: Comparisons of sequential procedures for selecting the best binomial population. Proc. Sixth Berkeley Symposium on Prob. Statist. (In Press).

Hoel, D. G., Sobel, M. and Weiss, G.: A two stage procedure for choosing the better of two binomial populations. Biometrika. (In Press).

Serial No.: NIEHS-B-006
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Probability Models and Competing Risks in the Study of Mortality Data"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop statistical techniques for assessing the effects of a treatment on specific diseases in survival studies.

METHODS EMPLOYED: A probabilistic model has been studied for describing the occurrences of particular diseases in a competing risk framework. Nonparametric techniques are used to estimate the model, giving both the disease incidence and cumulative mortality adjusted for competing causes of death. A nonparametric method of estimating non-lethal disease incidence has also been investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These methods are much more efficient in their use of experimental data than the classical interval techniques of mortality estimation which require much larger sample sizes. Also a clearer picture of mortality patterns is produced.

PROPOSED COURSE: A continuing effort will be made to develop new nonparametric procedures for application to survival data. Of particular concern is the need for methods of testing disease dependence.

PUBLICATIONS

Hoel, D. G.: A representation of mortality data by competing risks. Biometrics. (In Press)

Hoel, D. G. and Walburg, H. E.: Statistical analysis of survival experiments. J. Nat. Cancer Institute. (In Press).

Serial No.: NIEHS-B-007
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Epidemiological Study of Lung Cancer in Workers Exposed to the Inhalation of Zinc Chromate Paint in Airplane Construction and Maintenance Plants"

PREVIOUS SERIAL NUMBER: NIEHS-E-003

PRINCIPAL INVESTIGATOR: D. W. Gaylor, Ph.D.

OTHER INVESTIGATORS: M. D. Hogan, Ph.D. and Patricia B. Geiser, R.N., M.S.

COOPERATING UNITS: U. S. Air Force

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Epidemiological studies have shown that workers in chromate producing plants have an abnormally high incidence of cancer of the lung. Observations in Europe suggest that workers exposed to chromium pigments also experience a respiratory cancer hazard, but epidemiological evidence is lacking.

The airplane manufacturing and maintenance industry appears to offer an opportunity for studying workers who have been exposed to the inhalation of zinc chromate paints and related compounds. This follow-up study of persons who have been occupationally exposed to chromium compounds was designed to determine whether exposure to zinc chromate paint by inhalation results in a increased risk to lung cancer.

METHODS EMPLOYED: Arrangements were made with the U. S. Air Force to obtain the names of painters, whose employment had been terminated during the ten year period prior to July 1959, together with identifying information and a brief summary of each worker's employment at the base. For controls, similar data were obtained for workers in the same plant who presumably were not exposed to chromium. Unfortunately, no smoking histories were available for these workers. These data were collected by the Occupational Studies Unit, Epidemiology Branch, prior to July 1971. Then, upon transfer of most of the program of that unit to the National Institute of Occupational Satety and Health, the above data were sent to the Biometry Branch, NIEHS, for statistical analyses. Mortality

follow-up through 1966 has been completed by the Biometry Branch. Data on decedents' occupation, birth date, sex, race, exposure, age at death and cause of death have been prepared for computer input.

MAJOR FINDINGS: Statistical analyses have not been performed as yet.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study provides information regarding the epidemiology of respiratory cancer relative to zinc chromate paint exposure.

PROPOSED COURSE: Statistical analyses will be performed to determine the role, if any, of exposure to chromium by inhalation.

Serial No.: NIEHS-B-008
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Molecular Orbital Calculations on Some Polychlorinated Biphenyls and Other Biphenyl Derivatives"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: M. W. Anderson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

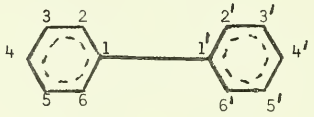
Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The major objective is to determine the electronic distribution in some polychlorinated biphenyls and other biphenyl derivatives. Another objective is to investigate the energy surface as a function of the dihedral angle between the two rings. This information will be used to help interpret the NMR spectra of these compounds and to help investigate the charge transfer complexing potential of the polychlorinated biphenyls.

METHODS EMPLOYED: The all valence electron CNDO/2 procedure in molecular orbital theory was used to perform the calculations. This is a semi-empirical procedure which has had success in predicting the correct trends in series of molecules.

MAJOR FINDINGS: Correlations between proton chemical shifts and electronic charge densities have been obtained for those compounds for which the NMR spectra are available. Calculations indicate that the rings of the polychlorinated biphenyls which have chlorine attached to the 2, 2', 6 and 6' positions



are very restricted in their rotational motion with respect to each other, i.e., the energy surface has a steep minimum with respect to the dihedral angle between these two rings. For the 4, 4' substituted biphenyls, the energy is essentially independent of the dihedral angle. This restricted rotation is also being investigated by temperature dependent NMR studies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROBLEMS OF THE INSTITUTE: The biological activity of the polychlorinated biphenyls could have some relationship to their ability to form charge transfer complexes. The energy surface studies are important because the ability of these biphenyls to bind to macromolecules or to undergo metabolism might depend upon the ease with which they can undergo conformational changes.

PROPOSED COURSE: Continued investigation of the charge transfer complexing property of the polychlorinated biphenyls and of the energy surface with respect to the dihedral angle between the rings is proposed.

Serial No.: NIEHS-B-009
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Investigation of Isokinetic Gradients for Rate-Zonal Centrifugation"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: M. W. Anderson, Ph.D. and D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: N. V. Hoesly

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The objectives are to develop a computer program which will construct an isokinetic gradient from the approximate boundary and initial conditions, and to investigate the effects of the assumption of an ideal particle (smooth and spherical) on the gradient profile.

METHODS EMPLOYED: In order to obtain an approximation of the effects of particle non-ideality on the isokinetic gradients, a factor which qualitatively accounts for non-ideality is introduced into the equation which constructs the isokinetic gradient profile. A comparison is then made between these gradients and those for an ideal particle. This method is necessary because the needed quantitative expressions are not available for non-ideal particles.

MAJOR FINDINGS: To obtain maximum separation of particles in a centrifugation experiment which employs an isokinetic gradient, it is essential to choose the correct boundary conditions. In particular, let S_{max} be the maximum value of the sedimentation coefficient which a particle can have and still remain within the gradient during the run. Then for two types of particles with sedimentation values S_1, $S_2 < S_{max}$, the separation, $r_1 - r_2$, is inversely proportional to S_{max}. Since S_{max} is a prescribed boundary condition, it should then be chosen as small as possible in order to obtain a maximum separation. In addition, a particular relationship between S_{max}, the rotor speed and the duration of the run must be satisfied. The effects of non-ideality can be very large on the isokinetic gradient profiles.

SIGNIFICANCE TO-BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Isokinetic gradients may give better separation of particle mixtures than the standard linear gradients, i.e., rough and smooth microsomes. Thus, it is necessary to theoretically investigate these gradients and develop computer programs to construct them.

PROPOSED COURSE: A comparison between the theoretical investigations and experimental results are necessary. A study of the predicted and experimental separations with latex spheres will be a first step in this comparison.

Serial No.: NIEHS-B-010
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Statistical Analysis of the Carcinogenic Potential of NTA"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. J. Connor, Ph.D.(NCI), D. G. Hoel, Ph.D., M. D. Hogan, Ph.D.

OTHER INVESTIGATORS: J. K. Haseman, Ph.D.

COOPERATING UNITS: National Cancer Institute

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: From May 1967 to May 1969, the Procter and Gamble Company conducted a feeding study of nitrilotriacetic acid (NTA) in the Charles River rat. A comprehensive review of the data generated by this study was undertaken by the Program and Data Analysis Unit of the Carcinogenesis Area, Etiology, of the National Cancer Institute. As part of this review, members of the Biometry Branch, NIEHS, collaborated in a statistical analysis of the tumor data.

METHODS EMPLOYED: In most of the analyses, the data was divided into two groups, (1) that generated from scheduled sacrifices and (2) that which arose from non-scheduled deaths. Because only one tumor was found among animals scheduled for autopsy before the fifteenth month of the study, all animals that died or were sacrificed prior to fifteen months were excluded. Treatment vs control differences in the proportions of various types of tumors as well as in all tumors combined, calculated for both the number of animals at risk and the actual number of animals autopsied, were examined for both male and female animals. In addition, the relationship between the onset of tumors and the severity of kidney lesions was considered.

MAJOR FINDINGS: Females autopsied during the 21-23 month period showed a significant increase in non-mammary tumors. For the visceral organ tumors taken collectively, there was a statistically significant ($P < .05$) increase in the treated animals. The question of whether or not there was an increase in the incidence of malignant tumors in the treated animals was not resolved

as the results for one pathologist gave a statistically significant increase and the findings for another pathologist resulted in no overall difference between treated and control animals. Finally, for both the male and female treated animals there appeared to be a positive relationship between kidney lesions and the occurrence of tumors. The interpretation of these results is complicated by the fact that autopsies were not performed for 17% of all animals surviving fifteen months and, in particular, for approximately 41% of the animals in the non-scheduled death group.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because NTA is proposed as a component of commercial laundry detergents and therefore may appear in the water supply, the relevance of the possible carcinogenic potential of NTA is critical.

PROPOSED COURSE: This project has been completed.

Serial No.: NIEHS-B-011
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Statistical Analysis of the Teratogenic Potential of NTA"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. W. Gaylor, Ph.D.

OTHER INVESTIGATORS: M. D. Hogan, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Data were assembled from experiments performed by the Bio-test Laboratories, Procter and Gamble, International Research and Development Corporation and NIEHS. A comprehensive statistical analysis of the teratogenic potential of nitrilotriacetic acid (NTA) was undertaken.

METHODS EMPLOYED: In all of the studies considered, the rat was used as the experimental animal. All but one experiment used oral feeding. NTA was administered in combination with MeHgCl, MeHgOH and $CdCl_2$. Statistical analyses were performed for maternal mortality, fetal mortality, specific anomalies and total anomalies.

MAJOR FINDINGS: The addition of NTA to MeHgCl produced no pattern of internal anomalies or fetal mortality. The addition of 40 mg/kg of NTA to 8 mg/kg of MeHgCl indicated an increase in edema and cleft palates. In a single study, no consistent deleterious effects were detected for NTA added to MeHgOH. When NTA was added to $CdCl_2$, with both administered subcutaneously, an increase in maternal and fetal mortality was observed. However, with oral administration, no deleterious effects were detected. There were certain experimental situations where NTA appeared to protect against cadmium and methylmercury toxicity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These results provide more information on the maternal and fetal toxicity and teratogenic potential of NTA.

PROPOSED COURSE: This project has been completed.

Serial No.: NIEHS-B-012
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Simulation of the Identification of Cases from a National Death Index"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: D. W. Gaylor, Ph.D. and M. D. Hogan, Ph.D.

OTHER INVESTIGATORS: P. B. Geiser, R.N., M.S.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The major objective is to establish the amount of information needed to identify subjects from a National Death Index.

METHODS EMPLOYED: A sampling of names from various telephone directories was used to estimate the probabilities of identical names and initials. Simple probability theory was employed to estimate the frequencies of identical birth dates, ages and partial Social Security numbers. Sex was not considered as an identifier since it results in only two categories.

MAJOR FINDINGS: For identification with a minimum of incorrect matching of death index entries to study group cases, the following information is needed when available from a death certificate:

> first name, middle name, last name (including maiden family name for females), birth date (mo/day/yr) and age, and state of birth.

The last 4 digits of the social security number would be highly desirable in the absence of any of the above information.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study provided an estimate of types of information (and of their relative importance) that would be needed for the efficient operation of the National

Death Index as an epidemiologic tool that many investigators feel is an absolute necessity in the study of chronic disease.

PROPOSED COURSE: This project has been completed.

Serial No.: NIEHS-B-013
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Effects of Fluorocarbon Injection and Inhalation of NiS, Ozonized Gas or Tobacco Smoke on Lung Cancer in Rats"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. W. Gaylor, Ph.D.

OTHER INVESTIGATORS: A. Ottolenghi, M.D. and P. B. Geiser, R.N., M.S.

COOPERATING UNITS: Pathologic Physiology Branch

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the effects of the inhalation of NiS, ozonized gas and tobacco smoke (with and without fluorocarbon injection) on the incidence of lung cancer in rats.

METHODS EMPLOYED: The experiment was performed by the Hazelton Laboratories for the National Cancer Institute. Lung tissue slides were re-examined at NIEHS by the Pathologic Physiology Branch. Lung cancer was considered confirmed if carcinoma was observed by NIEHS or if at least adenoma was observed for a previously reported carcinoma. The only animals included were those which survived at least 400 days for which acceptable lung tissue slides were available. Both sexes of Charles River CD rats were used. In addition to controls, animals were exposed to NiS, ozonized gas or cigarette smoke by inhalation. Half of the animals received an injection of hexachlorotetrafluorobutane. Half of the injected animals received the fluorocarbon before exposure to NiS, ozonized gas or cigarette smoke, and the other half received the fluorocarbon injection 4-6 weeks after the commencement of exposure.

MAJOR FINDINGS: The injection of fluorocarbon produced no detectable effect in this study. A comparison of the NiS group with the controls shows an increase in the proportion of animals with lung cancer which is significant at the $P \leq .01$ level:

NiS	14/189
Ozonized Gas	1/191
Tobacco Smoke	1/198
Controls	2/189

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These results provide more information on the etiology of lung cancer.

PROPOSED COURSE: This project has been completed.

Serial No.: NIEHS-B-014
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Nonparametric Tests in Biometry"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: K. M. Patel, Ph.D. and D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: J. L. Carver

MAN YEARS

Total:	1.3
Professional:	0.0
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To develop nonparametric tests (1) for interaction in factorial experiments and (2) for testing equality of means in a completely randomized design against ordered alternatives when responses are subject to arbitrary right censorship.

MAJOR FINDINGS: (1) Assuming the linear models for responses, a nonparametric measure of interaction of two factors has been developed and the Wilcoxon-Mann-Whitney statistics are used in estimation of the measure. This has resulted in a nonparametric technique for detecting the presence of interaction in factorial experiments. (2) Using Breslow's results, a nonparametric technique has been developed where the distributions of the censoring variables are allowed to vary with the populations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The test procedures are useful in biomedical experiments where the treatments comprise a series of doses of substances and nothing can be assumed about the responses except that they are continuous. The aim of such experiments may be to determine whether responses are independent of the combination of the dose levels or to determine whether responses tend to be ordered according to dose levels when responses may be censored or not.

PROPOSED COURSE: Because of experimental blocking, nonparametric methods are required for testing equality of treatment means in a randomized block design against ordered alternatives.

Serial No.: NIEHS-B-015
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Statistical Tests for Goodness-of-Fit"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: K. C. Rao, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Often it is of interest to determine if experimental data were sampled from a particular distribution. If the assumed distribution is known, then three statistics (Kolmogoroff, Cramér-von Mises and chi-square) are recommended for tests of goodness-of-fit of experimental data. However, if the hypothesized distribution is characterized by some unknown parameters (e.g., the normal distribution of unknown mean and variance), then these parameters have to be estimated from the data. In such cases, the three above statistics cannot be directly employed unless a table of critical values is available appropriate to the hypothesized distribution. Because it is both mathematically and computationally difficult to construct such tables, research was undertaken to develop a technique so that existing tables can be used when some of the parameters are not specified.

MAJOR FINDINGS: A random variable which is a function of the observed data was developed for use with the Kolmogoroff, Cramér-von Mises and chi-square statistics. The limiting distributions of these statistics can be used for goodness-of-fit tests when some of the parameters are unknown.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fitting experimental data to specific distributions is a common biometry problem. These results improve the existing techniques for prediction.

PROPOSED COURSE: The project has been completed.

Serial No.: NIEHS-B-016
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Statistical Analysis of DDT, 2,7FAA and Piperonyl Butoxide Survival Experiments"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: P. B. Geiser, R.N., M.S.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The General Foods Corporation conducted a feeding experiment for the National Cancer Institute to assess the carcinogenic activity of piperonyl butoxide alone and in conjunction with pesticides and known carcinogens. Two survival studies were performed on albino rats. The first study combined doses of piperonyl butoxide and 2,7FAA, while the second study considered piperonyl butoxide and the p,p'-isomer of DDT.

METHODS EMPLOYED: Nonparametric techniques (see NIEHS-B-005) were used to test treatment effects on the prevalence of both non-neoplastic and neoplastic conditions. General survival was also examined.

MAJOR FINDINGS: Both studies showed an increase in bile duct hyperplasia and benign liver neoplasms in the females at the high dose (20,000 ppm) of piperonyl butoxide. In the DDT study, among the 100 day sacrificed females, an increase in pituitary hyperplasia was found at the high dose (5 ppm) of DDT. The 2,7FAA study showed increased incidences of malignant tumors and general life shortening at the high dose (100 ppm) of 2,7FAA.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of their use in the environment, the chemicals were studied for possible adverse health effects.

PROPOSED COURSE: This project has been completed.

OFFICE OF THE ASSOCIATE DIRECTOR FOR
SCIENTIFIC INFORMATION AND COMMUNICATION

OFFICE OF THE ASSOCIATE DIRECTOR FOR
SCIENTIFIC INFORMATION AND COMMUNICATION
Summary Statement

Scientific Liaison with Categorical Environmental Programs

As the organizations of the Environmental Protection Agency (EPA) and the National Institute for Occupational Safety and Health (NIOSH) have become established, liaison has become progressively easier. Relationships are now better than at any time in the previous five years, with the disappearance of old resentments and suspicions. The acquisition of program information has been greatly assisted by the assignment from OST, through the Ad Hoc Committee on Environmental Health Research, of responsibility for making a survey of relevant activities throughout the Federal programs. We now have on file virtually all of the Project Descriptions and about 95% of program information in this field. But retrieval of specific information, other than that reported to the OST Committee, will remain cumbersome until such time as semi-automatic handling is established. The report on the survey has gone to the Ad Hoc Committee, and will constitute an attachment to the Committee's report to OST-CEQ in May 1972. A group of scientists at the Branch Chief level from FDA visited for exchange of information. Similar meetings are being arranged for groups from CDC and Edgewood Arsenal. Communication at the policy level has been greatly facilitated by participation in inter-departmental activities such as the Ecology and the Environmental Health Research Committees of OST and CEQ, discussions with UK representatives, and conferences on topical matters, such as PCBs.

Quick Retrieval System for Scientific Information

The situation as reported last year is unchanged except for a greater storage of information and a sharpening of the procedures. The complete system in its Phase I form is now in operation. Information entry is from selected documents on to aperture cards in three forms: (a) typed citation, short abstract, key words, coded key words; (b) punched key words; and (c) micro-film of original document. Storage is in two forms: (a) manually in the cells of a coordinate (stressor x focus-of-interest) file; and (b) serially by entry number. Retrieval of small amounts of specialized information is manual from the coordinate file, of larger amounts by punch card collation from the serial file. At present, the latter is done remotely by telephone to N. C. Central University. Priority in entering material is now shifting to the project information obtained in the survey for OST. A thesaurus permits use of standardized key words and their 5-letter code equivalents.

Intramural Scientific Information Service (including Library)

This continues as a sectional activity directly responsible to the Associate Director. A Chief for an Information Branch which would have inclusive responsibility for Storage and Retrieval, Information Services and Compilation has not been recruited. The scope of the Library continues to expand to meet new intramural scientific activities. An automatic title service to intramural scientists is now in partial operation. Inter-library "loans" continue at high levels.

Scientific Information Evaluation and Reporting Service

The major effort has been completion of three of the four books on various aspects of environmental health planned in conjunction with the Fogarty International Center, and on detailed editing of a book on "Pulmonary Reactions to Coal Dust" largely in conjunction with the National Institute of Occupational Safety and Health (NIOSH). Proceedings of a small symposium on The Epidemiology of Heat Effects will appear in Environmental Research. Editorial responsibilities for an Institute quarterly publication, Environmental Health Perspectives, is shared with Dr. Falk. This Office will have responsibility for editorial work on the Report of the OST-CEQ Ad Hoc Committee.

Beryllium Case Registry

The Registry entries have been reviewed in terms of more stringent and uniform criteria. The value of a recognized central focus of information on berylliosis was demonstrated by a recent exposure episode in which the Registry staff was asked to make a disinterested evaluation. NIOSH has been asked if it would take over responsibility for the Registry's operation, since the contained information is highly relevant to its operation.

International Programs

The only program in which this Office is actively involved at the moment is the bilateral arrangement with France. A report is awaited from Dr. Sadoul, Gr. de Recherche de Physio-pathologie Respiratoire at Nancy, suggesting future exchanges. Possible bilateral arrangements with Australia have been suggested. We are assisting the New York Academy of Sciences in planning an international symposium on Pulmonary Effects of Organic Materials.

Editorial Committee

The number of papers submitted for clearance increased to 86 over the period July 1, 1971, to April 5, 1972.

Serial No.: NIEHS-OSI-001
1. Office of Associate Director for Scientific Information & Communication
2. Liaison
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Scientific Liaison with Categorical Environmental Health Programs"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-01

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: Categorical Environmental Health Programs

MAN YEARS

Total:	1.2
Professional:	0.4
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: The primary objective is to maintain awareness of relevant scientific and technical activities between the intramural operations of NIEHS and other Federal environmental programs. Formal processes support, but do not satisfy ultimate objectives of liaison -- meeting together, thinking together, working together.

BACKGROUND: One of the reasons advanced for creating NIEHS was the necessity for a facility which would provide the more basic, longer-term, multiple factor research necessary to the development of criteria, standards, and control measures by the categorical environmental health programs. This necessitates continuing exchange of information on problems, needed research, investigative activities, and emergent scientific information between the research and categorical activities. The need was expressed in announcements by the Surgeon General on the implementation of NIEHS, and has been the subject of frequent inquiry by OMB and DHEW representatives.

MAJOR ACCOMPLISHMENTS: Time has resolved most of the difficulties in establishing effective liaison with other Federal programs dealing with environmental health. Representatives at the policy level meet together with increasing frequency on various matters. A survey of Federal activities made for OST has opened up channels to 7 programs with major concern and 15 with smaller concern. A virtually complete set of relevant Project Descriptions is on file. Scientists

at the bench level are becoming increasingly acquainted and communicate directly. Visiting groups increase in frequency.

PROPOSED COURSE: Improved retrieval of information from stored Project Descriptions. Collaboration with any arrangements made by OST for continued periodic survey of Federal programs. Increased scientist - scientist communication.

Serial No.: NIEHS-OSI-002
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Quick Retrieval System for Scientific Information"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-02

PRINCIPAL ADMINISTRATOR: Richard K. West

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	1.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is the development and operation of a system for quick retrieval of information on scientific topics relevant to NIEHS programs.

BACKGROUND: The relatively unstructured nature of information obtained from categorical programs (see Project OSI-001) and other sources imposes the need for some system of retrieving information on a particular subject with speed and precision. The preparation of position and other staff papers (see Project OSI-005) also demands quick access to important relevant information. Intramural scientists need assistance in securing references, abstracts, and original documents relating to topics of immediate interest. It is desirable that one system be established for handling information acquired to meet these internal needs.

MAJOR ACCOMPLISHMENTS: Storage of topical information has been continued as far as staff shortages permit. A Project Reference Wall Board has been set up facilitating access to descriptions of activities by other Federal programs on topics of specific interest.

PROPOSED COURSE: Entry into QRS information contained in Project Descriptions obtained in the course of the OST survey (See OSI-001). Installation of automatic sorting machine. Cooperation with OST-sponsored surveys.

Serial No.: NIEHS-OSI-003
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "NEHSC Library"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-03

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	0.2
Other:	1.8

PROJECT DESCRIPTION

OBJECTIVES: To provide necessary library holdings and services for the use of NEHSC personnel.

BACKGROUND: Although the three neighboring universities provide excellent backup library resources, and the services of both the NIH library and the National Library of Medicine are available to NIEHS, a moderate library facility is essential in the immediate vicinity to minimize the time spent by scientists in travel and absence from active work, and to facilitate consultation of relevant material.

MAJOR ACCOMPLISHMENTS: Space in present quarters in Building 10 is saturated. Plans for organization of more space have been prepared. There are now 198 periodicals regularly received. Demands for interlibrary loans average 504/month. The annual purchase budget is $21,000. The staff received a group award for services rendered to Institute personnel.

PROPOSED COURSE: Move to larger space in Building 10 when available. Increased coverage as new Branches are established in the Institute.

Serial No.: NIEHS-OSI-004
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Intramural Scientific Information Service"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-04

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	0.8
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The objective is to provide relevant scientific information by both routine and demand services to NEHSC personnel.

BACKGROUND: Both the preparation of position and other staff papers, and the consideration of relevant scientific literature by working intramural scientists, requires the acquisition, assembly, and referencing of documentary information from a wide range of sources. Both the Scientific Directorate and the intramural scientists require assistance in (a) using the Quick Retrieval System (Project NIEHS-OSI-002); and (b) going beyond the limited internal library resources (Project NIEHS-OSI-003) to external sources.

MAJOR ACCOMPLISHMENTS: Information Services now constitutes a Section in the functional organization of OSIC, charged with securing such scientific information as is required by Institute members for the various purposes. This includes normal library operations (Project NIEHS-OSI-003), personal services according to profiled requirements, and meeting demands for special purposes. It provides an essential input to the growing demand for position papers on a variety of subjects (Project NIEHS-OSI-005). Much of the information secured becomes potential input to the QRS (Project NIEHS-OSI-002).

PROPOSED COURSE: Increased services with access to additional external sources.

Serial No.: NIEHS-OSI-005
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Scientific Information Evaluation and Reporting Service"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-05

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	0.4
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objective is to evaluate the relative significance of various environmental stresses and preparation of reports on selected topics in environmental health.

BACKGROUND: a. Efficient allocation of Institute resources to specific investigations presupposes a knowledge of the range of environmental situations that affect man, the relative seriousness of their effects, the feasibility of ameliorating the results, and the extent of investigative attention already being given to the significant situations.

b. The Directorate is frequently called upon to present position papers on various environmental health matters of national import.

MAJOR ACCOMPLISHMENTS: Three of the four books developed in conjunction with the Fogarty International Center have been published, as has the book on Pulmonary Responses to Coal Dust. Proofs of Discussion at the NYAS Conference on Coal Workers Pneumoconiosis have been revised. Another Conference on Pulmonary Reactions to Organic Materials has been planned. Dr. Lee represents DHEW on the CEQ-FCST Committee on Ecology and is serving as Executive Secretary of the OST-CEQ Ad Hoc Committee on Environmental Health Research. (See also OSI-001) An Institute quarterly publication, Environmental Health Perspectives, has been initiated. The first issue, comprising papers presented to a PCB Conference held by NIEHS, was published in May 1972.

PROPOSED COURSE: Complete the fourth FIC book. Continue with the quarterly publication. Participate in the NYAS Conference on Organic Materials. Complete work on the Ecology and Environmental Health Research Committees. Cooperate with any continued OST actions. Begin editorial work on APS Handbook of Environmental Physiology.

Serial No.: NIEHS-OSI-006
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Beryllium Case Registry"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-06

CONTRACTOR'S PROJECT DIRECTOR: H. Kazemi, M.D., Chief, Pulmonary Unit

PROJECT OFFICER (NIEHS): Douglas H. K. Lee, M.D., Ph.D.

COOPERATING UNITS: NIOSH

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to maintain the Beryllium Case Registry originally set up in 1952 by Dr. Harriet Hardy, providing for: (a) periodic appeal to physicians to report all suspected cases of beryllium poisoning; (b) examination of reports by an expert panel to decide admission to the Registry; (c) acquisition of additional evidence of diagnostic significance; (d) diagnostic assistance to the reporting physician; (e) periodic follow-up on progress of cases.

The importance of maintaining the Registry as an information source lies in: (a) inadequate knowledge of late disease manifestations; (b) changing patterns of exposure to beryllium; and (c) uncertainties about the basic patho-physiological processes.

MAJOR ACCOMPLISHMENTS: The Registry has 817 cases listed, of whom 540 are known or believed to be alive. Some difficulties in completing the 1969 follow-up have been resolved. A revalidation of old entries has been completed, and stricter rules are applied to the admission of new cases. There were 11 new cases added in 1971 and 27 specimens were examined for Be. All radiographs have been re-examined on a uniform basis. Sixty physicians attended a meeting on BCR and berylliosis. Members of the BCR team assisted in a study of an apparent exposure incident in a Be plant.

PROPOSED COURSE: Continue operation of BCR. Start another triennial follow-up of old cases.

Serial No.: NIEHS-OSI-009
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

PROJECT TITLE: "Participation in International Programs"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-09

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.1
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The objective is to participate in those aspects of international programs operating through DHEW that are relevant to the NIEHS mission.

BACKGROUND: With the transfer of the categorical environmental programs out of DHEW, NIEHS becomes a mainstay of DHEW interests in environmental health. This applied particularly to international programs, in which environmental aspects are getting increasing attention. Close cooperation is maintained in this connection with the Fogarty International Center and with the Office of International Health.

MAJOR ACCOMPLISHMENTS: A four-week tour was arranged for Dr. Sadoul, Gr. de Recherche de Physio-pathologie Respiratoire at Nancy, visiting under INSERM arrangements. A report was received from Dr. J. Wyatt on his stay in Nancy.

A program on carcinogenesis and mutagenesis set up under US-Japan Cooperative Medical Sciences Program has been organized with Dr. de Serres as Chairman.

Two meetings have been held with UK counterparts on establishing a bilateral cooperative program.

Discussions on possible bilateral arrangements have been held with the Scientific Counsellors of the Australian Embassy.

Several foreign scientists have visited in connection with scientific meetings.

PROPOSED COURSE: Complete arrangements for scientist exchanges with France. Cooperate with other developments as necessary.

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

INSTITUTE DIRECTOR'S SUMMARY STATEMENT ON EXTRAMURAL PROGRAMS

NIEHS during the past year made further progress in its efforts to bring extramural research and training awards into closer alignment with the Institute's human-oriented mission. Some of the grant commitments acquired by the developing Institute through their transfer from the former Bureau of State Services were of marginal relevance to the research and training mission of NIEHS. In the ensuing period, this Institute has maintained the dual function of honoring support commitments to these grantees while endeavoring to phase them out either through orderly termination or by assisting in their reassignment to more appropriate support agencies.

Attainment of these objectives generally is complete in the individual research grant program. Realignment in the training grant program is less advanced because of the relatively longer commitment periods involved and because of the inherent complexities of training in the environmental health field. A substantial number of active training grants are scheduled for competing review during the current year and phase-out of the less appropriate programs may be expected to continue.

Excellent new applications have been received and funded by NIEHS during the past year. A significant number of other proposals were recommended for approval but were not activated because of fiscal limitations. It is clearly unfortunate that excellent environmental health research proposals by highly qualified scientists have been lost to the Institute. However, the NIH system of peer review and priority ranking has continued to provide a firm basis for selection of the very best among competing grant applications.

Because NIEHS may guide but not direct the preparation of research grant applications, there is often a time lag between the identification of emergent environmental health problems and the initiation of grant research upon them. The university-based Center program has been very useful for providing prompt responses to new environmental questions. Part of the flexibility designed into the Center programs derives from the availability of core funds which may be used to initiate early research feasibility studies on new environmental problems. This concept thus enables the NIEHS to suggest timely new efforts in environmental health research.

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS
Summary Statement

RESEARCH GRANTS

The number of research grant applications assigned to NIEHS in fiscal year 1972 increased by 63% over last year. Although NIEHS Extramural Programs was able to effect a small increase in the number of individual research projects supported, fiscal limitations permitted the awarding of only 43% of the new and competing renewal applications that were recommended for approval by advisory review groups. Approximately 126 grant projects were supported, with total funding of $8.7 million. Among these grants were 30 for new research projects.

TRAINING PROGRAMS

Thirty-one training program awards were made to teaching institutions in fiscal year 1972. Supporting funds totalled $3.1 million. These figures represent a substantial decrease from the number of training programs supported in the previous year, although the total funds remain about the same. This, in part, reflects the increased costs of support to training programs. It also indicates the preferential support of large multicategorical programs in institutions that provide a broad range of training opportunities in environmental health research. NIEHS support of university-based training programs provides to highly qualified students the fundamental knowledge of concepts and procedures necessary for productive research careers in the environmental health sciences. There are a few grants of peripheral relevance to the Institute training mission remaining in the program. These grants were acquired by transfer during the early days of the Institute and have been maintained as moral commitments. Nine training grants were phased out during the past year in favor of new programs which will be more responsive to the national need for trained environmental health scientists.

Total funds of $264,000 were awarded for support of 10 postdoctoral and special fellowships and 8 Research Career Development Awards. Approximately 68% of these funds went to support the RCDA's, 2 of them new this year. Postdoctoral fellowship and career development awards continue to be important mechanisms for meeting the uncommon requirements peculiar to preparation of environmental specialists. Training in traditional departmental disciplines often has not provided the broad and comprehensive research background necessary for thorough understanding of environmental science complexities. Individual fellowship and Career awards provide advanced multidisciplinary exposure to environmental health concerns and techniques; they permit early implementation of specific Institute programs, and their flexibility affords an effective response to the expanding national needs for highly qualified environmental research scientists.

UNIVERSITY-BASED CENTERS

National Institute of Environmental Health Sciences university-based centers provide a unique milieu for the investigation of environmental health problems.

During the past year NIEHS began support of a project to study the effects of environmental lighting on the metabolic fate of nutrients, drugs and toxins. The investigator is determining, for example, the molecular effects of artificial lightwaves on riboflavin and vitamin A, compounds which can act as photosensitizers to alter the metabolism of other chemicals in the skin. Research in this field is relatively new but its implications in human health are clear. Fundamental data from studies such as this will provide a basis for understanding the beneficial and harmful properties of solar and artificial lighting.

TOXICOLOGY, GENERAL

NIEHS provides grant support for initial publication costs of a series of essays on toxicological subjects. The series is aimed at students and other persons who may wish to expand their understanding of toxicological principles and techniques. Each essay is prepared by an expert selected from a particular field of toxicology, and is written at a level technical enough to be authoritative, but which informed laymen can understand. Two volumes have been published to date and have been made available in "soft back" to broaden their accessibility to students. These publications are of particular importance because they will extend the appreciation of toxicology concepts at a time when the nation is experiencing an increased awareness of toxic environmental problems.

A new grant has been funded to investigate the venom of the brown recluse spider. The bite of this spider causes a chronic necrotizing wound in humans and has caused death in children. The scientist will identify the specific toxins in the venom, will explore their toxic and biochemical mechanisms and will attempt to discover an effective therapeutic antivenom. The investigator has collected a supply of venom and is now conducting early studies of its molecular, enzymic and toxic properties.

Many of the chemical compounds of immediate concern in environmental health sectors contain chlorine atoms. The chlorinated hydrocarbon, DDT, is widely known. Enzymatic degradation of these compounds is a reaction basic to pharmacologic and toxicologic concepts. The process sometimes leads to intermediates more toxic than the parent molecule. A grant-supported scientist is studying the fundamental mechanisms of enzymic dechlorination to further describe known metabolic pathways, to search for new ones and to compare their relative importance in selected animal species. Data from this research will have broad implications in environmental and occupational health, and it may provide a stronger basis for predicting the toxicity of new chlorinated compounds.

Alpha chloro-ether compounds are widely found in the chemical industry. Some of them are highly reactive alkylating agents. An NIEHS grantee investigator is exploring the carcinogenic potential of certain alpha chloro-ethers and has shown that rats exposed to them at low airborn levels develop nasal and pulmonary carcinomas. The scientist will continue these studies to further assess the human health implications of these findings.

their metabolic reactions and their variability among insect species. Work to date has been done on chlorinated hydrocarbon detoxification mechanisms in the housefly. The investigator also has recently begun a study on the influence of insect hormones on these enzyme functions. Information derived from this project will relate to questions of selective toxicity and insect resistance. It will also advance the design of insect control measures that are efficient in pest management but minimally disruptive to other organisms.

NIEHS supports a program project for study of pesticide chemistry and toxicology. This coordinated research program consists of a community of individual research efforts, all related to some aspect of chemical pest control. The projects focus generally into three areas: analysis and instrumentation, toxic mechanisms and the environmental fate and effects of pesticidal compounds. Serious efforts have been made under this grant to understand, for instance, the toxic mechanisms that have been associated with reduced wildlife populations in this country. These and the other projects in the program represent an integrated effort to find new and safer measures for pest control.

PHYSICAL FACTORS

Temperature and Humidity

NIEHS supports a program grant devoted to the multidisciplinary study of physical factors in the environment and the complex responses to them in normal animals and man. Within this program scientists have investigated combinations of heat, cold, humidity, solar radiation and other environmental factors to detail their effects on biological systems and subsystems. They have measured the effects of clothing, exercise and other complicating parameters. They have studied neural, cardiovascular, respiratory and endocrine responses evoked to counter these stresses and maintain body temperature. The group recently has expanded studies on behavioral temperature control and comfort responses to temperature change. New exposure and measuring techniques developed under this grant will permit more accurate quantitation of thermal stresses and biological responses to them. Sophisticated data processing developments will facilitate the integration of these specific data into broader aspects of biological temperature regulation. The importance of the program nature of this grant lies in its integrative function--physical, physiological and psychophysical parameters of temperature regulation being investigated in one combined research effort.

Radiation Hazards

NIEHS is supporting a research project on the mechanisms of adverse photosensitivity reactions in skin, the influence of temperature and humidity on these reactions and the biologic difference in persons suffering them. The investigator has been probing whether the skin reactions are phototoxic or photoallergic in nature. His work is at the molecular level in the cellular and subcellular structures of the skin. This scientist is also testing whether individuals with inborn metabolic errors can be detected by a rapid screening procedure. There is much to be learned in this field, particularly as photosensitivity episodes become increasingly associated with new use of chemicals in soaps, cosmetics and related consumer products.

have been shown to cause serious toxicoses and cancer in animals and humans. It is important to identify the responsible food-borne toxins, to determine which microorganisms produce them and to measure the environmental conditions favoring their production. It is equally important that means be devised for detecting these metabolic toxins in stored foods. Finally, it is essential that their toxic mechanisms and potential be explored.

NIEHS supports a number of research projects on these and related food toxicity problems. One investigator has studied aflatoxin biosynthesis in Aspergillus cultures. Once he has determined the chemical sequence in the formation of these toxins, the scientist will evaluate the modifying effects of temperature, oxygen, fungicides and other external factors on the synthesis. Another scientist worked with Rubratoxin-B, a specific mycotoxin, to determine its acute and chronic toxicity in mammals, its mechanisms of toxicity and detoxification and its pathologic manifestation in animal systems.

METALS

The clinical manifestations of many overt heavy metal intoxications are well documented. The fundamentals of trace metal metabolism, interactions and subclinical toxicity, however, are much less understood. In the past year NIEHS began support of a grant to determine the biochemistry, physiology and toxicity of abnormal trace metals. The investigator is exploring the interactions of trace metals at the cellular and subcellular levels and the mechanisms by which metals and their combinations disrupt physiologic functions of growth, longevity, reproduction, etc. He is attempting to learn the proximal role of certain heavy metals in the etiology of human chronic diseases.

Chelating agents are compounds that by binding with metal ions mobilize them for excretion from the body. They have found application in the clinical diagnosis and treatment of metal poisoning. NIEHS supports several projects concerned with chelating agents. Among them is a grant specifically devoted to lead chelation mechanisms. The scientist has studied in detail the sites and magnitude of lead mobilization by chelating agents and their efficacy in releasing body stores of the metal. In addition, he will assess the toxicity of the lead chelation products themselves.

PESTICIDES

Biochemical studies of metabolic detoxification systems have improved the basis for predicting enzyme induction and inhibition changes in response to combinations of drugs, pesticides and industrial and environmental chemicals. These studies, however, often have been conducted at exposure levels higher than would be encountered under normal circumstances. NIEHS supports a research project to detect and measure the potential for interaction among combinations of environmental stressors at low concentration levels. The investigator has developed a method for biochemically screening simultaneous and sequential combinations of chemicals by measuring their ability to alter detoxification enzyme functions. Data from this project will provide new information on environmental synergists and further insight into enzymatic detoxification mechanisms in animals and man. Another, closely related, project has been funded to explore the subcellular enzyme systems of insects,

RESEARCH HIGHLIGHTS

These descriptions of selected NIEHS grant projects are representative of the extramural research activities in FY-1972.

AIR POLLUTANTS

An imposing variety of the stressors appropriate to environmental health concern become to some extent airborne and, thus, readily gain entry to the body through the respiratory system. A large portion of the NIEHS grant program is devoted to study of airborne gases, vapors and aerosols to determine their molecular properties in the atmosphere, their distribution and fate in the respiratory tract, their toxic properties and mechanisms and the biologic defense mechanisms against them.

An NIEHS grantee is testing the postulate that ozone, nitrogen dioxide and other oxidants produce their toxic effects in part by disrupting the lipid chemistry of biological membranes and that an antioxidant such as vitamin E may be used to protect the lung and blood cells from membrane damage. The study is answering questions concerned with environmental etiology of chronic lung diseases, including emphysema, and may produce basic data on the aging process as well.

The Institute funded a new research project concerned with the molecular and psychophysical bases of odor perception, intensity, masking and adaptation. Data from this study will expand understanding of olfactory mechanisms and their protective function in detecting noxious environmental conditions.

Macrophage cells in the pulmonary air sacs serve an important cleansing function by disposing of inhaled foreign particles deposited in the lung. An NIEHS grantee is studying these cells to learn details of their function and has assessed the detrimental influences of tobacco smoke, oxidants and other air pollutants on this protective mechanism.

FOOD TOXICANTS

Food Additives

Nitrosamine compounds have been shown to cause cancers in certain animal species. They perhaps are formed in the body by the interaction of nitrites and nitrogen oxides with secondary amines. NIEHS supports a project for research on a nitrosamine compound--nitrososarcosine--to describe its synthesis in the body, to assess its acute and chronic toxicity, to explore its mechanisms of carcinogenesis and to evaluate its potential hazard as a mutagenic agent. This project, which is still in its early stages, further illustrates the potential hazards that lie in the interaction of innocuous environmental chemicals to form toxic compounds.

Natural Products

Storage of foodstuffs and feed is often accompanied by fungal infestation and molding. Metabolic products of certain fungi, when ingested with these foods,

These centers are designed to integrate the multifaceted research of scientists from a variety of disciplines. Communication of ideas across research groups and scientific disciplines is important in the function of a center. Thus, the interrelationships of environmental problems with each other are explored by interacting research approaches. The stability of a center program and its opportunity for comprehensive study provide a highly effective resource for the research training of highly qualified young people. Their preparation for careers in the environmental health sciences gives them an early appreciation of the complexity inherent in many environmental science concerns. NIEHS supported six Centers in FY-1972 with total funds of $3.5 million.

The Environmental Health Sciences Advisory Council's Subcommittee for University-based Centers continued to conduct important liaison between the program interests of NIEHS and its advisory committees and the research and training staffs of the centers. Through periodic visits to each of the Environmental Health Science Centers, the Subcommittee has maintained effective communication of mutual administrative and scientific concerns and has provided general assurance that the center research and training programs maintain substantial relevance to the mission of NIEHS.

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